INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE & TECHNOLOGY
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www.vmsindia.org

Isolation, Identification and Anti-bacterial Activity of Chromobacteria Isolated from Various Sources

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Abstract
Pigmented bacteria are also known as chromobacteria. Pigment is molecule that have colour. Bacteria produce pigments for various reasons and it play an important role. Various growth mediums can be used to isolate different types of bacteria. However due to the high cost using synthetic medium, there is a need to develop new low cost process for the production of pigments as well as during the isolation procedure.

The microorganism such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcenscens*, *Kocuria turfensis*, *Micrococcus luteus* and *Micrococcus roseus* etc produce large number of pigments and they were isolated from various sources.

The antibacterial activity was studied on several organism like *B. subtillis, S. aureus, E. coli, S. typhi, K. pneumonia*, among these five pathogens the pigment was found to be more effective against gram positive and gram negative bacteria.

Keywords: Pigment, Chrmobacteria, Antibacterial activity. Various sources.

INTRODUCTION

Bacteria which produce pigment are called as chromogenic bacteria. Some bacteria produce pigment as part of their normal metabolism including black, white, brown, golden, silver, florescent green, yellow or blue. They display all color of the rainbows. The specific color of the pigment is characteristic for bacterium, pigmented bacteria will form cultures that exhibited some color. *Pseudomonas aeruginosa* will produce green yellow pyocyanin pigment, *Staphylococcus aureus* produce golden yellow Staphyloxainthin pigment. *Serratia marcenssens* produce brilliant red prodigiosin pigment, *Kocuria turfensis* produce orange betacarotene pigment. *Micrococcus roseus* produce pink canthaxanthin pigment and *Micrococcus luteus* produce yellow colored carotenoid pigment.

Today, pigment are used in many areas such as medicine, animal feed, paper, ink, food, textile. Pigment production is very useful for bacteria. There are many reason for pigment production to produce the pigments photosynthesis, UV protection, Defense mechanism, secondary metabolites for storage of energy. Some pigment are active againt phytopathogens and human pathogens. Extremophiles are very colorful. Pigment of extermophiles required for respiratory and photosynthetic function. Different types of pigment extracted from microbe. Chemically bacterial pigments are pyrole, phenozyne, carotenoid, xanthophylls, quinine and quinine derivatives. It has been proved that only aerobic and facultative anaerobic bacteria are pigmented because molecular oxygen is essential for pigmentation. Therefore anaerobic bacteria are non-pigmented. (Rokade M. T. and Dr Pethe A. S. 2016)

Health environment concerns due to unmonitored utilization of synthetic colorants revived interest in natural dye as they are safer, healthier, biodegradable, and exhibit higher compatibility with the environment. (Raju V. N. and Radha T 2015). The advantage of pigment production from microorganism include easy and fast growth in the cheap culture media (Bhat *et al.,* 2013). Pigment are compound with characteristics of importance to many industries. In the food industry they are used as additives, color intensifiers, antioxidants etc. pigment come in a wide variety of colors, some of which are water soluble while another non-soluble in water (Tibor 2007). Microorganism are known to produce a variety of pigment, therefore they are promising source of food colorants (Aberoumand A 2011) Natural pigments possess anticancer activity, contain pro-vitamin A and have some desirable properties like stability to light, heat and pH (Joshi *et al.*, 2003)

Color is an integral part of both human culture and in human life. Colors have been used to enhance the aesthetic value of everyday human life. It is the most important characteristic of food, clothes in everyday life. (Samyuktha S. And Mahajan S. N 2016). In the past, consumers did not care about the kind of pigment used in food coloring. But with reference to food colorants

recently there is an aversion towards synthetic pigment owing to the belief such as "synthetic pigment are associated with several illnesses" and "natural pigments have pharmacological benefits" (Clydesdale F 1993) Color which are added in food are based on anthocyanin which are derived from natural source like red grapes or beet but first additive color were synthetic dyes. (Sharma D 2014)

Natural pigments not only have the capacity to increase the marketability of products but they also display advantageous biological activities as antioxidants and anticancer agents (Malik *et al.*,2012) Pigment of various color are synthesized to protect the cells of microorganism from injurious effect of light rays of visible and near ultraviolet range. (Rashid *et al* 2014). The various types of pigments produced by microorganism are pyocyanin, staphyloxainthin, prodigiosin, betacarotene canthaxanthin and carotenoid. Among them all carotenoid are the most widely observed and studied pigment. Carotenoids are nothing but lipid soluble classes of molecules associated with the lipid function which are sensitive to oxygen, heat, and light (Ciapara *et al.,* 2004)

METHOD

Collection of sample

For isolation of chromogenic bacteria, various sample are required. For isolation of *Pseudomonas aeruginosa* sewage sample and soil sample were used. The soil sample was collected from different area of Akola city. Sewage sample were collected in screw cap bottle. *Staphylococcusaureus* was isolated by using pus sample and soil sample. Pus sample were collected from pathological laboratory. *Serratia marcescens* obtained from soil and sputum sample. So for the isolation of *Serratia marcescens* sputum samples collected from pathological laboratory. *Kocuriaturfensis* was isolated from milk and milk product *Micrococcus roseus* and *Micrococcus luteus* were isolated from soil samples.

Media

The media used for enrichment and isolation of pigmented bacteria were Nutrient Agar, Nutrient broth and Mueller-Hinton Agar which were obtained from Himedia, India.

Isolation of Pigmented Bacteria

For isolating media Nutrient agar plus 2% glycerol with pH 7.2 was used. The plates were then incubated at 37^{0}C for 24 to 72 hours. Only the pigmented bacterial colonies were selected and sub-cultured on the nutrient agar plates for further studies.

Identification of Pigment Producing Bacteria

Gram staining and microscopic study were performed for the isolation of pigmented culture from nutrient agar plates. The biochemical tests performe. Indole test, Methyl Red (MR), Voges Proskauer (V) and citrate. Enzyme test Amylase, Gelatinase, Oxidase and Catalase tests were performed. Identification of isolates obtained in pure culture was based on Gram staining, morphology, growth characteristics on selective and differential Media and biochemical test.

Purification of Pigment Producing Bacteria:

The morphological characteristics of plates with discrete pigmented colonies were isolated and recorded. For pure culture, the morphologically-selected isolated colonies were picked up from the plates and sub-cultured as streak plate method. 3-6 isolated bacterial colonies were picked with the help of sterile inoculating needle and streaked on nutrient agar plate (with glycerol) for pure culture. After overnight incubation at 37°C, morphological characteristics of the colonies were recorded.

Extraction of pigments

The bacterial isolates were first grown for 24 h followed by centrifugation at 6000 rpm for 30 min. both the supernatant and bacterial cell pellets were extracted using either 95% methanol or 99.5% acetone in the ratio of 1:5 (supernatant) or until the pellet was colorless. The bacterial pellet was then discarded while the supernatant was extracted using ethyl acetate followed by concentration using rotary evaporator. The pigment concentration process was carried out until around 1% of the initial solvent volume was left in the evaporation flask. The concentrated pigment was then transferred into glass petri dishes prior to drying for 3 days at 60 ^{0}C.

Anti-bacterial Activity of the Pigments

The antibacterial activity of the pigments was tested by Agar-Cup Diffusion Method. 20 ml of Mueller Hinton Agar was poured into the Petri-dish. The test was performed by swabbing of the growth inhibition zone of the plate and then swab was streaked onto Mueller Hinton agar plate and 7 mm well bored in the agar. 100µL of extracts was poured into the wells. The plates were incubated for 24 h at37°C and the zone of inhibition was measured in mm.

Observation:

Table1: Colony characters of isolated bacteria on nutrient agar media after 24 hrs at 37^{0}C were observed as follows.

Name of Isolate	**Isolate no. 1**	**Isolate no. 2**	**Isolate no. 3**	**Isolate no. 4**	**Isolate no. 5**	**Isolate no. 6**
Size Shape	1 – 2 mm Roughly circular	2-4 mm Circular	0.5 –1 mm Circular	1– 2 mm Circular	5-3.5 mm Circular	5-3.5 mm Circular
Color	Bluish green	Golden yellow	Red	Orange	Yellow	Pink
Margin	Flat	Smoot	Smoot	Smoot	Smoot	Smoot
Elevation	Irregular	Convex	Convex	Convex	Convex	Convex
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Gm. Character	Gram – ve. Rod	Gram + ve Cocci	Gram - ve Cocobaccili	Gram + ve Cocci	Gram + ve. Cocci	Gram + ve. Cocci
Motility	Motile	Non-Motile	Motile	Non-Motile	Non-Motile	Non-Motile
Confirmed Isolates	*P. aureginosa*	*S. aureus*	*S. marscensces*	*Kocuria turfensis*	*M. luteus*	*M. roseus*

Table2: Anti-bacterial activity of the pigments extracted from isolated pigment forming bacteria.

Isolate no.	**B. subtillis**	**S.aureus**	**E. coli**	**S.typhi**	**K. pnemoniae**
1	21 mm	23 mm	19.5 mm	16 mm	20.5 mm
2	21 mm	29.5 mm	26 mm	24.5 mm	53.5 mm
3	24 mm	15.5 mm	28 mm	12 mm	17.5 mm
4	30 mm	25 mm	18.5 mm	24.5 mm	21.5 mm
5	27 mm	30 mm	27.5 mm	39.5 mm	14 mm
6	11 mm	26.5 mm	11 mm	13 mm	9.5 mm

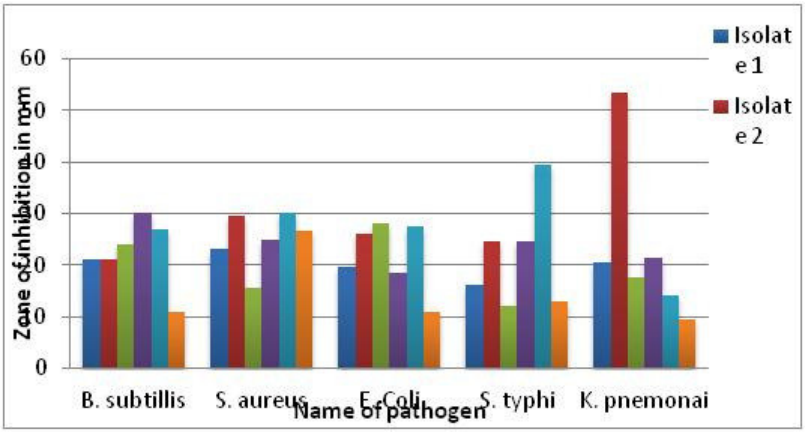

Fig1: Graphical representation of antibacterial activity

RESULT AND DISSCUTION

Microbial pigments are a promising alternative to other color additives extracted from vegetables or animals because they are considered as natural, pose no seasonal production problems and show high productivity.

Pseudomonasaeruginosa was isolated by using sewage sample and soil sample. *Pseudomonasaeruginosa* was showing Gram-ve Coccobacilli motile bacteria. On nutrient agar dark green colony was found, on pseudomonas isolation agar yellowish greenish fluorescent colony was found. We have found in sugar fermentation tests, Glucose, Lactose, Mannitol, Fructose, Sucrose, Ribose, Dextrose sugar fermentation an Acid and gas produdction. In IMViC tests Citrate +ve. In enzyme test, this

isolate was showing Catalase +ve, Gelatinase +ve, Oxidase +ve, Urease +ve Amylase –ve test.

For isolation of *S. aureus* pus and soil sample were used. *S. aureus* was showing Gram+ve cocci non motile bacteria. On nutrient agar yellow color colony was found, on milk agar golden yellow color colony was found. As per biochemical characteristics we have found in sugar fermentation test Glucose, Lactose, Mannitol, Fructose, Sucrose, Ribose, Dextrose sugar fermentation and acid and gas production. In IMViC MR +ve, Citrate +ve, VP +ve. In enzyme test this isolate were showing Gelatinase +ve, Urease +ve, Catalase +ve, Amylase +ve test.

Serratia marscensces commonly obtained from Soil and Sputum sample. *Serratia marscensces* was showing Gram –ve cocco bacilli motile bacteria. On nutrient agar red color colony was found. As per biochemical test Glucose, Lactose, Mannitol, Fructose, Sucrose, Ribose, Dextrose, sugar fermentation also acid and gas production. In IMViC tests Citrate +ve. In enzyme test this isolate were showing Gelatinase +ve, Urease +ve, Oxidase +ve, Catalase +ve.

Kocuria turfensis were isolated from Milk and Milk product. *Kocuria turfensis* was showing Gram +ve Cocci non motile bacteria. On nutrient agar orange colored colonies were observed. As per biochemical test Glucose, Lactose, Mannitol, Fructose, Sucrose, Ribose, Dextrose, sugar fermentation on acid and gas production. In IMViC tests Indol +ve, Citrate +ve. In enzyme test this isolate were showing Urease +ve, Oxidase +ve, Catalase +ve. Amylase +ve was found.

Micrococcusluteus were isolated from soil sample. *Micrococcusluteus* was showing Gm+ve cocci non motile bacteria. On mannitol salt agar yellow colored colonies were observed. As per biochemical test Glucose, Lactose, Mannitol, Fructose, Sucrose, Ribose, Dextrose, sugar fermentation al acid ansod gas production. In IMViC tests Citrate –ve, In enzyme test this isolate were showing Urease +ve, Oxidase +ve, Catalase +ve was found.

Micrococcus roseus were isolated from soil sample. *Micrococcusroseus* was showing Gm+ve cocci non motile bacteria. On mannitol salt agar pink colored colonies were observed. As per biochemical test Glucose, Lactose, Mannitol, Fructose, Sucrose, Ribose, Dextrose, sugar fermentation also acid and gas production. In IMViC tests MR +ve, Indol +ve. In enzyme test this isolate were showing Urease +ve, Oxidase +ve, Catalase +ve. Amylase +ve was found.

The present study indicates that pigment production is influenced by physical factors such as temperature and pH of the culture medium. There should be many other factors, affecting pigmentation by the bacterium such as source and concentration of nutrient components.(Rashid Md. M 2014). The optimum condition for pigment production was studied, pH 7, glycerol- 2%, temperature-37, Carbon source 0.5% mannose, time 72hrs and NaCl 0.5%

During investigation total 50 samples were analyzed from soil, water, pus, sputum, Urine, blood taken for different pigment producing bacteria were obtained, out of which 32 samples are positive for pigment production.

Staphyloainthin pigment was found to be more significantly effective against all tested pathogen species such as *K. pnemonia* produced (53.5 mm) diameter for zone of inhibition, *S. typhi* (24.5mm) *E. coli* (26mm), *S. aureus* (29.5mm) and *B. subtilis* (21mm). In the present study, bacterial pathogen *K. pnemonia* was found to be highly inhibited by the pigment extract of *S. aureus* with the zone of inhibition 53.5 mm. The pathogen like *S. aureus* and *S. typhi* were more effectively inhibited by the pigment extract *micrococcusroseus*. (30 and 39.5 mm). Antibacterial activity showed that there was no uniform response among bacterial strains in susceptibility can be attributed to differences in cell wall composition. The reason was referred to be difference in the structure of the cell walls. (Singh *etal.*, 2007). Selective antibacterial activity may be due to several factors, including the charge density, structure of lipopolysaccharides and lipid composition of the cytoplasmic membrane in Gram-negative and Gram-positive bacteria (Devine and Hancock, 2002). Our results indicated that the chemistry of the pigments has significant influence on its antimicrobial activity.

CONCLUSION

This study deals with the isolation, idenfication and antimicrobial activity of chromobacteria from various sources at suitable pH, Temperature, Carbon source, and Medium. The emergence of strains of bacteria resistant to common antibacterial agents has become one of the most important problems in clinical medicine. The search for new antibiotic is always on. As the reports for pigments having antibiotic like activity are rapidly increasing, they should be studied for selective toxicity so that they can be produced commercially for human use.

ACKNOWLEDGEMENTS

The author thankful to Prof.and Head Dr Archana S. Pethe and Principal Dr. Shubhash Bhadange Shri Shivaji College of Arts, Commerce and Science for providing infrastructure facilities and their constant support for this study.

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