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ISOLATION AND IDENTIFICATION OF CHROMOGENIC BACTERIA FROM VARIOUS SOURCES.

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ABSTRACT

Pigments are molecule that have colour. Some bacteria produce pigment as part of their normal metabolism including black, white, brown, golden, silver, florescent green, yellow or blue. The specific colour of the pigment is characteristic for bacterium, pigmented bacteria will form cultures that exhibited some colour. They display all colors from rainbows. The microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcenscens*, *Sarcina maxima*, *Micrococcus luteus*, *Micrococcus roseus*, etc. produce large number of pigments and they were isolated from various sources. An ideal pigment producing microorganisms should be capable of using a wide range of Carbon and Nitrogen source, have tolerance to pH, temperature and minerals and give reasonable color yield.

KEYWORDS: Chromogenic bacteria, Bacterial pigment.

INTRODUCTION

Bacteria which produce pigment are called as Chromogenic bacteria. They are only few in number. In present investigation *Pseudomonas aeruginosa* produce green-yellow phenazine pigment, *Staphylococcus aureus* produce either golden yellow or lemon yellow pigment. *Sarcina maxia* produce orange pigment *Serratia marcenssens* produce brilliant red coloured prodigiosin pigment. *Micrococcus roseus* produce pink pigment and *Micrococcus luteus* produce yellow coloured pigment.

Some pigments are water soluble they leak out of cells and diffuse through the water-based culture medium. This causes the medium to change colour. Other pigments are water insoluble they remain within the cells, so only the colony becomes coloured. Thus we conclude that when the microbial cells, are used to produce the colour the term refers to as Microbial Pigments, Different types of pigment extracted from microbes. Chemically bacterial pigments are pyrole, phenozyne, carotenoid, xanthophylls, quinine and quinone 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.

Why do some bacteria produce pigments?

Pigment production is very useful for bacteria. Pigment production in bacteria is associated with morphological characteristics, cellular activities, pathogenesis, protection and survival. Pigments of photosynthetic bacteria carry out photosynthesis like plant chlorophyll. Pigments produce by bacteria to absorb U.V. radiation. Some pigments are active against phytopathogens and human pathogens. Extremophilles are very colourful. Pigments of extermophiles required for respiratory and photosynthetic function. Pigment confers antibacterial and heavy metal resistance. Pathogenic Staphylococci are multidrug resistant because of their pigment which acts as a barrier for antibiotics acting on cell wall and plasma membrane.

Colours are one of the significant visual properties of food and coloring of foods has been an age old practice. This practice has amplified many folds with the invention of synthetic colorant principally due to their physical properties of good stability and coloring ability (Pattnaik *et al.*, 1997). Despite the fact that the commercial market is ruled by synthetic pigments some of them may be toxic, carcinogenic or many cause severe damage to vital organ (Duran *et al.*, 2002). Because of this, a strong interest is naturally coloring alternative is needed. As compared to other available synthetic pigments, natural pigment from microbial source are potentially good alternative one, carotenoid are a group of coloured terpenoids with antioxidant properties which are widespread in the plants and animal kingdom, as well in fungi and in photosynthetic and non photosynthetic microorganisms (Phadwal 2005).

Recently carotenoids supplements are used commercially as food colorants, animal feed and more recently for

nutraceuticals, cosmetics and pharmaceutical purpose (Klein-Marcuschemer *et al.,* 2007). In addition carotenoid have attracted superior attention as compared of human health. Caretenoid can inhibit various types of cancer and it enhances the immune response (Guerine *et al.,* 2003). These pigments are capable of quenching photosensitizing interacting with single oxygen (Krinskyet *et al.,* 1994) and scavenging proxy radical (Coon *et al.,* 1992) It also protect "life style related" such as cardiovascular disease and age related muscular degeneration due to their antioxidant activity and provitamin A function (Stevenet *et al.,* 2000) They play an important role in protection of muscular region of the retina and hence prevention of cataracts and increase level of iron absorption (Mares *et al.,* 2002)

METHOD

Collection of sample

For isolation of chromogenic bacteria, various samples are required such as Sewage, Sputum, Pus, Soil, Milk and milk product were used. *Pseudomonas aeruginosa* was isolated by using sewage sample and Soil sample: the sample was collected from campus of Shri Shivaji College, Akola. Sewage sample were collected in 250ml bottle. For isolation of *Staphylococcus aureus* Pus and Soil sample were used. Pus sample were collected from pathological laboratory, which were brought into the the laboratory and used for isolation of *Staphylococcus aureus. Serratia marcenscens* commonly obtained from Soil and Sputum sample. So for the isolation of *Serratia marcenscens* above samples collected and brought into laboratory for the isolation of *Serratia marcens. Micrococcus roseus* and *Micrococcus luteus* were isolated from soil sample. *Sarcina maxima* was isolated from milk and milk products such as Cheese, Butter, and Khoya.

Screenig Of By Enriched Technique

Soil sample were taken and inoculate separately in Nutrient broth and Tubes were incubated for at 25-30°C for 48 hrs. Then loopful from incubated Nutrient broth was streaked on Pseudomonas isolation agar, plates of this agar incubated at 28°C for 48 hrs. Pigment producing colonies were observed on pseudomonas agar. These pigmented colonies were inoculated on Glycerol peptone agar and incubated at 37°C, for 72 hrs. Strong pigment production that is Greenish coloured is observed on glycerol peptone agar.

Isolated cultures were identified through implementation of Phenotipic characters, such as Gram's Staning, Colony Characters like Sugar Fermentation, IMViC test, were studied. Among the enzymes Catalase, Oxidase, Gelatinase, Amylase, Casienase and Urease tests were performed. Soil and Pus sample were inoculated on Nutrient agar and incubated at 37°C for 24 hrs. Next day Golden yellow, coloured colonies were observed these colonies were inoculated on different agar plates. Such as Baired parker agar, Mannitol salt agar, simillarly Gram's staning and cultural characters were studied. For motility the colonies were inoculated on Nutrient broth tube. Coagulase test was done and Biochemical and IMViC test were performed. *Serratia marcensces* were also obtained from the soil sample producing brilliant red coloured colonies. Nutrient agar plates inoculated with soil sample were kept at 37°C for 24hrs, and the same plate after 3-4 days red coloured colonies were obtained. Simillarly Gram's Staining is done and Biochemical and IMViC test were performed.

Sarcina maxima were isolated from Milk and milk product such as cheese, butter, khoya etc. Above sample were inoculated on Nutrient agar plates, plates were kept for incubation at 37°C for 3-4 days, and orange coloured colonies were obsereved. Simillarly Gram's staning, Motility is done and Biochemical characters and IMViC test were perfomed. *Micrococcus luteus* and *Micrococcus roseus* were isolated from soil. Soil sample was inoculated on Mannitol salt agar plates were incubated at 37^{o}C for 48 hrs., then after incubation *Micrococcus luteus* shows yellow colonies and *Micrococcus roseus* shows pink colonies. Simillarly Gram's staning, Motility is done,and Biochemical characters and IMViC test were performed.

FACTERS AFFECTING ON PIGMENT PRODUCTION

1) Temperature

To study the effect of temperature on pigment production, isolated bacteria were streaked on Nutrient agar plates by four ways streaking and some plates were kept at 37°C, Some plates kept at 7-10°C, some plates were kept at 20-25°C. Then after 24hrs it was observed that maximum pigment produce at 37°C.

2) pH

pH also influences the pigment production for this Nutrient agar plates with different pH(3,5,7,9,11) were prepared. Isolated bacteria were streaked on it. And plates were kept at suitable temperature depending upon the pigment produce by bacteria. Maximum pigment production was observed at pH 7.

3) Medium

Generally medium containing 1% glycerol enhances the pigment production for this peptone glycerol agar plates were prepared and all necessary condition for pigment production were provided.

SOLUBILITY OF PIGMENT

Most of the bacterial pigment is soluble in water. Some, of them soluble in distilled water and we found that some of them were soluble in organic solvent like chloroform, ether, acetone and alcohol.

Sr. No.	Pigment Produced by Bacteria	Solvent
1	Pyocyanin	Distilled water
2	Prodigiosin	Benzene + Ethanol
3	Sarcinaxanthine	Distilled Water
4	Lipoprotein	Tap water

OBSERVATION AND RESULT

Colony characters of isolated bacteria on nutrient agar media after 24hrs at 37c were observed as follows.

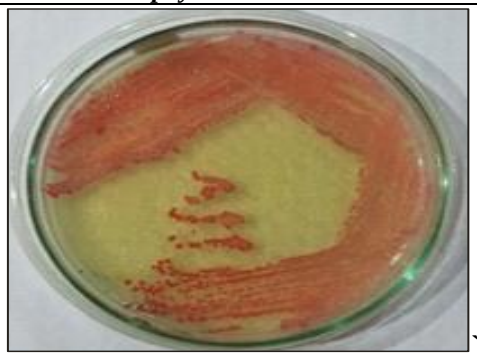
Sr. No.	Name Of Isolate	Size (mm)	Shape	Colour	Margin	Elevation	Opacity	Morphology	Motility
1	*Pseudomonas aeruginosa*	1-2	Roughly Circular	Bluish green	Flat	Irregular	Opaque	Gm-ve rod Non capsulated	Motile
2	*Staphylococcus aureus*	2-4	Circular	Golden yellow	Smoot	Convex	Opaque	Gm+ve Cocci arranged in cluster.	Non-Motile
3	*Sarcina maxima*	1-2	Circular	Orange	Smooth	Convex	Opaque	Gm+ve Cocci	Non - Motile
4	*Serratia marscensces*	0.5-1	Circular	Red	Smooth	Convex	Opaque	Gm-ve cocco bacilli	Motile
5	*Micrococcus luteus*	5-3.5	Circular	Yellow	Smooth	Convex	Opaque	Gm +ve Gm+ve Cocci arranged in tetrad	Non-motile
6	*Micrococcus roseus*	5-3.5	Circular	Pink	Smooth	Convex	Opaque	Gm+ve Cocci arranged in tetrad	Non-motile

Photo Plate of isolated chromobacteria

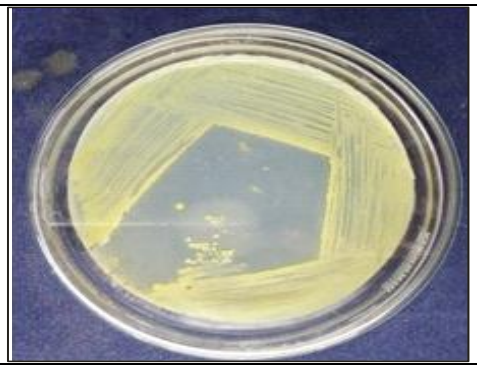
Pseudomonas aeruginosa

Staphylococcus aureus

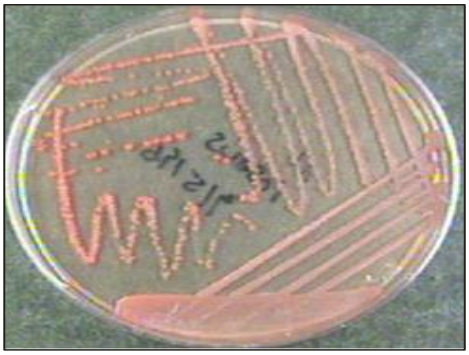
Sarcina maxima



Serratia marcescens



Micrococcus luteus



Micrococcus roseus

IMViC ClASSIFICATION OF ISOLATES.

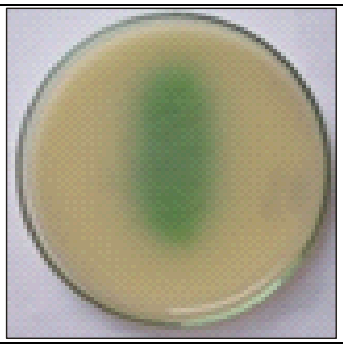
Sr. No.	Name of Isolate	Indole	Methyl Red	Voges Pauskaur	Citrate
1	*Pseudomonas aeruginosa*	- ve	-ve	-ve	+ve
2	*Staphylococcus aureus*	-ve	+ve	+ve	+ve
3	*Sarcina maxima*	+ve	-ve	-ve	+ve
4	*Serratia marscensces*	-ve	-ve	-ve	+ve
5	*Micrococcus luteus*	-ve	-ve	-ve	-ve
6	*Micrococcus roseus*	+ve	+ve	-ve	-ve

ENZYME TEST OF ISOLATES

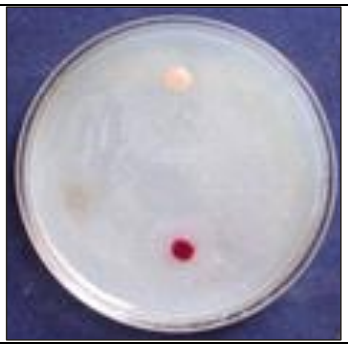
Sr. No.	Name of Isolate	Amylase	Casienase	Gelatinase	Oxidase	Urease	Catalase
1	*Pseudomonas aureginosa*	-ve	+ve	+ve	+ve	+ve	+ve
2	*Staphylococcus aureus*	+ve	-ve	+ve	+ve	+ve	+ve
3	*Sarcina maxima*	+ve	-ve	-ve	+Ve	+ve	+ve
4	*Serratia marcescens*	-ve	-ve	+ve	+ve	+ve	+ve
5	*Micrococcus leteus*	-ve	-ve	-ve	+ve	+ve	+ve
6	*Micrococcus roseus*	+ve	-ve	-ve	+ve	+ve	+ve

Photographs of Enzyme positive test

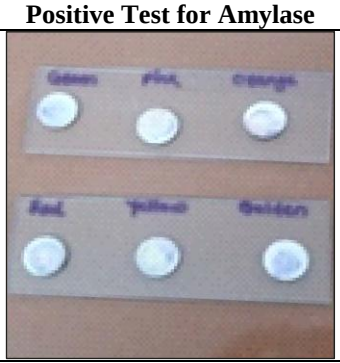
Positive Test for Amylase



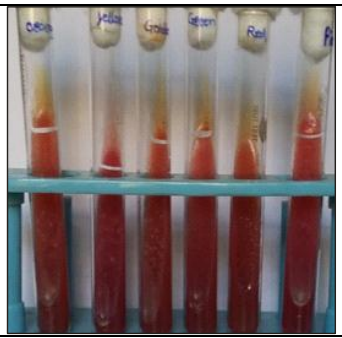
Positive Test for Caseinase



Positive Test for Gelatinase



Positive Test for Oxidase



Positive Test for Urease

Positive Test for Catalase

Sugar Fermentation Test

Sr. No.	Name of Isolate	Glucose		Fructose		Mannitol		Sucrose		Ribose		Dextroe		Maltose		Lactose	
		A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
1	*Pseudomonas aeruginosa*	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
2	*Staphylococcus aureus*	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	*Sarcina maxima*	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
4	*Serratia marscenscens*	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
5	*Micrococcus luteus*	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-+ve	+ve	+ve
6	*Micrococcus roseus*	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve

RESULT AND DISSCUTION

During investigation total 92 samples were analyzed from soil, water, pus, sputum, Urine, blood, Milk and milk product taken for different pigment producing bacteria were obtained, out of which 79 samples are positive for pigment production All of them were observed for colony characters at 37°C for 24hrs.(Table 2). From these pigment producers 3 were diffusible and 3 were non diffusible (Table 1). (Keneni & Gupta 2009*;*Raj D.N.*et al* 2009*.)* On the basis of Gram's staining, motility, colony characters and biochemical test like sugar fermentation test and enzymes test isolates were identified as *Pseudomonas aureginosa, staphylococcus aureus, Sarcina maxima, serratia marcenscens, Micrococcus luteus,* and *Micrococcus roseus.* The isolation of carotenoid producing microbes from some abnormal environment condition was also reported by Arunkumar *et. al*(2006) Natural colours or bio colour have neumerous applications in numerous sectors as an alternate towards the use of biocolours which find its applications in numerous sectors as an alternate for synthetic chemicals. Further the natural pigment obtained in this study would be tested for its application in all field.

CONCLUSION

This study deals with the isolation and identification of chromobacteria from various sources at suitable P^H, Temperature and Medium. The results shows the pigments can be produced in laboratory also pleasant to see. Hence, the pigments have the potential to use as food colorants, Decorating glassweres, colouring cotton textiles and having remarkable antibacterial activity against multidrug resistance pathogens. However, further research is needed before using these pigments as food colorant.

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