

Isolation and Characterization of Dye Producing Microorganisms from Soil Sample

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Abstract: *Microorganisms are the most important tools in biotechnology to produce variety of molecules like enzyme antibiotics and pigments microorganisms are a promising source for natural colours • The presence of pigments has been reported in entire microbial world including bacteria ,fungi yeast, algae, and protozoa Industrial production of natural pigment by microbial fermentation has • several advantages such as cheaper production easier extraction and higher yields throughout the years In the food industry they are used as colouring agent and antioxidants, pigment, are present in various colors.*

Keywords: *Microorganisms.*

I. INTRODUCTION

Microorganisms are the most important tools in biotechnology to produce variety of molecules like enzyme antibiotics and pigments microorganisms are a promising source for natural colours • The presence of pigments has been reported in entire microbial world including bacteria ,fungi yeast, algae, and protozoa Industrial production of natural pigment by microbial fermentation has • several advantages such as cheaper production easier extraction and higher yields throughout the years In the food industry they are used as colouring agent and antioxidants, pigment, are present in various colors Caretenoids are fat soluble highly unsaturated red, orange, and yellow pigments that are naturally present in plantThe green coloured pigment are produced by the species pseudomonas this are used in textile industry it is naturally found in environment this is toxic in nature Prodiagnosin is red colour pigment primarily reported from serratia marscens prodiagnosin was named after its extraction from bacillus prodigious and later given the Violacin is a violet or purple colour pigment The objective of our study was to isolate and characterise pigment producing bacteria from different soil samples. These pigment are extracted and used as natural colorants for cloth dyings, fungi, bacteria and algae

AIM AND OBJECTIVE

- Microorganism are used in dye production process in many industries like textile, food the microorganisms have ability to produce pigment and making extraction of this pigment for dye
- In this context this work aimed at “Isolation and Characterization of Dye producing Bacteria from soil Sample” This was taken with following objectives
- Collection of soil sample in different area
- Isolation of pigment producing bacteria
- Study of colony characters and morphology characters of isolates • Study the biochemical test of the isolates
- Prepare extract of isolates pigment
- Antibacterial agent are used to remove toxicity

II. MATERIALS AND METHODS

Isolation of pigment producing bacteria: - Various sample like soil in different types were collected soil samples were collected from different areas of vita city. Such as balwant college campus in different sections like red soil, black soil in college area All the samples are collected in a dry and clean container, from this pigment producing bacteria were isolated and used for the present study 1 gram of each sample was mixed with 9 ml of saline 9(0.85% of Nacl W/V) The mixture was vortexed for uniform suspension from the a loopful of suspension was inoculated in nutrient agar plate by using four



quadrant streak plate technique. The inoculated plates were incubated at 37 °C for 24 hours from this orange and green colour pigment producing bacteria was selected and repeatedly sub cultured for pure culture

1. Identification of bacteria: - Identification of bacterial isolate was performed by morphological characteristics and biochemical tests

Morphological characteristics :- • Colony characterization of pigment producing bacteria from nutrient agar plate was done by colony size, shape, margin, opacity, consistency, elevation, motility and gram-staining

Biochemical Test: - • Biochemical test performed were Indole test, methylred test(MR), voges proskauer(vp), sammon citrate test, oxidase test, ureas test, catalase test, gelatin decomposition test and nitrate reduction test

Screening of pigment production:- • The colour pigment producing bacteria was taken from the nutrient agar for pigment production. A loopfull of culture was inoculated in to sterile 100ml nutrient broth mixed with 2% glycerol and incubated at 37°C for 2 days in a rotary shaker

Extraction of pigment: - • The pigment producing bacterial culture added in nutrient broth for extraction of pigments incubated this broth 37°C for 2 days in a rotary shaker centrifuge at RPM for 2 hours after centrifuge collect the supernatant and add alcohol then add oxalic acid as a binding agent for dye production

III. RESULT AND DISCUSSION

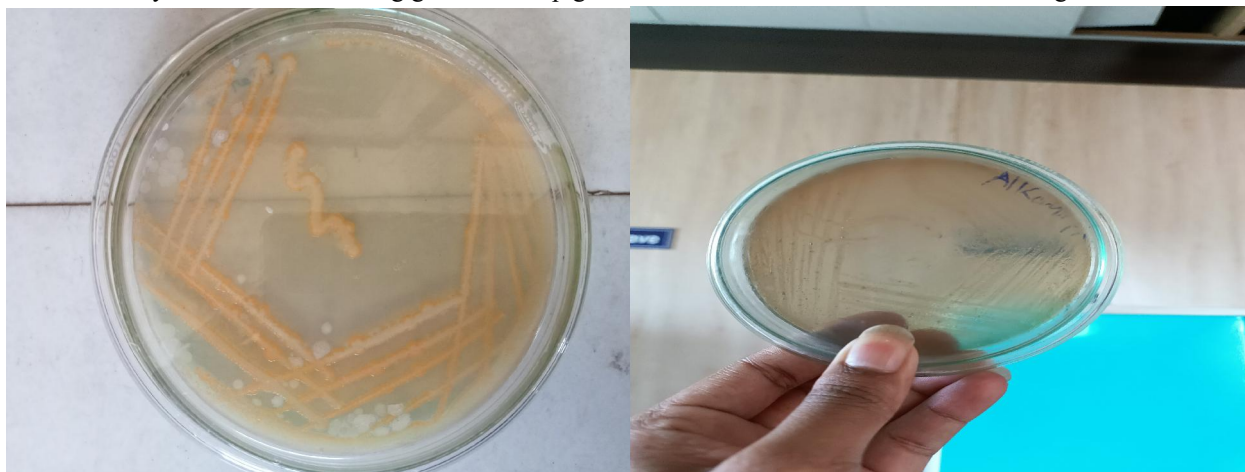
3.1 Isolation of pigment producing bacteria:- • Various samples like soil in different areas were taken for isolation of bacteria among the colonies of bacteria formed on the nutrient agar orange and green colonies are produced these orange and green colour colonies are repeatedly sub cultured in nutrient agar and centramide agar to produce pure culture

3.2 Identification of bacteria:- • Orange and green, color bacterial isolates were grown on nutrient agar identification of bacteria were done by performing morphological and biochemical tests

3.2.1 Morphological characterization:- • Characterisation of orange and green pigment producing bacteria from nutrient agar plate was done by colony shape colour, margin, opacity, consistency, elevation and gram staining mentioned in table Morphological Characteristics

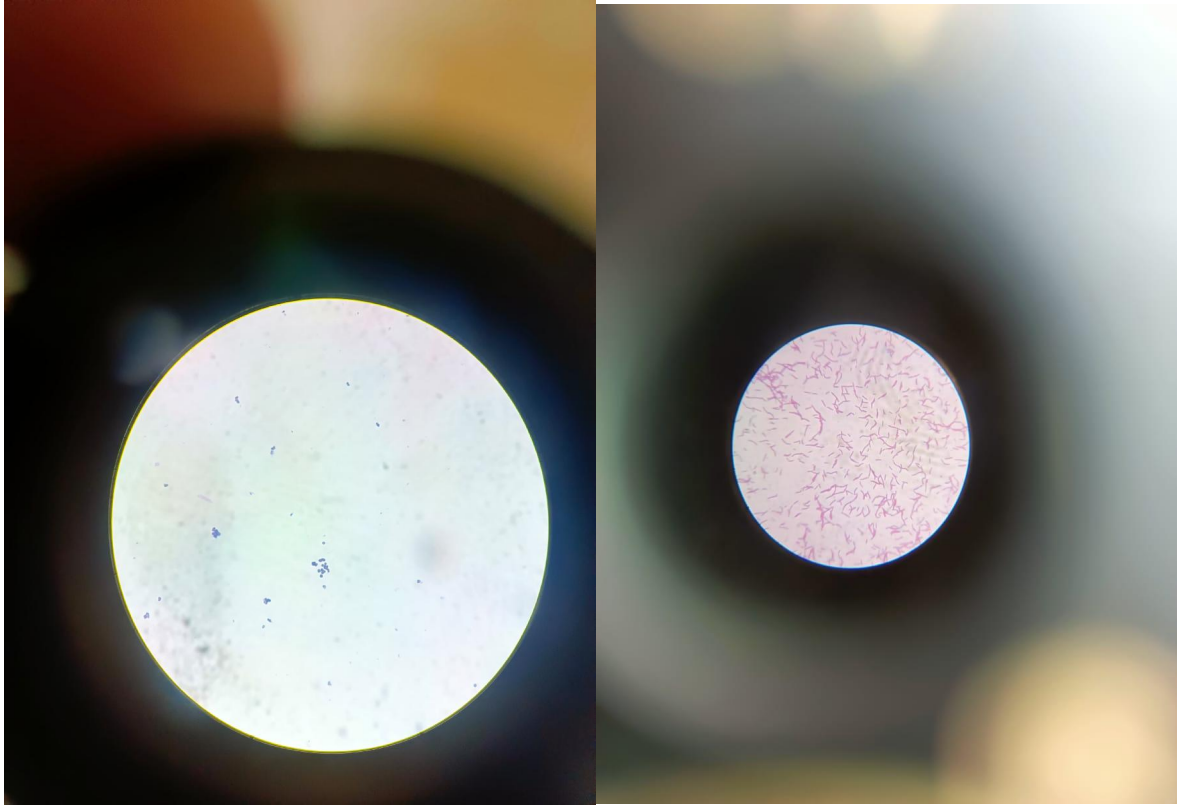
From the above observation orange pigment isolates was found to be exiguobacterium species and green pigment isolates was found to be pseudomonas species

3.3 Screening and Extraction of pigment from pigment producing bacteria • Isolated pigment were grown in nutrient agar broth for 2 days at 37°C for screening green colour pigment was extracted from nutrient broth using different techniques



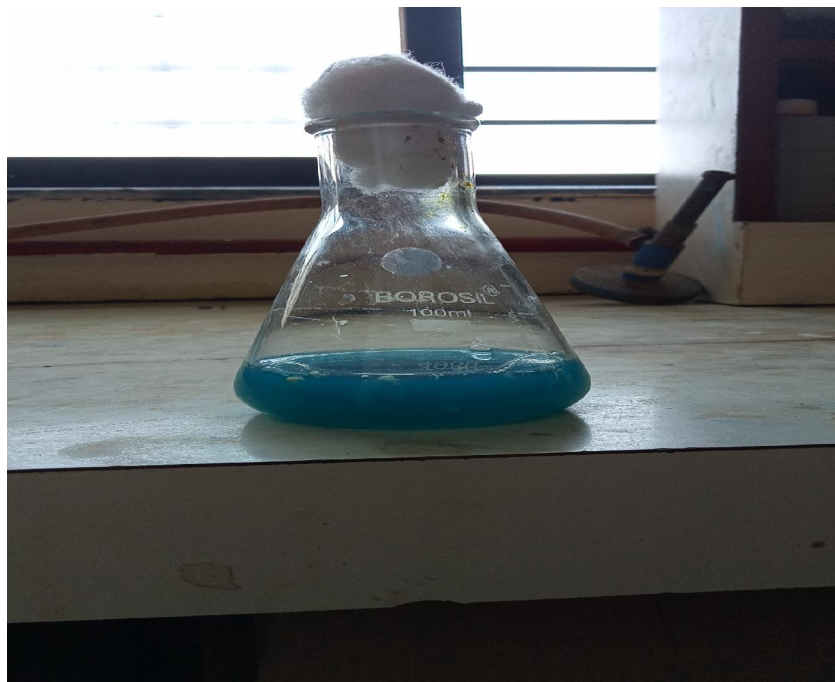
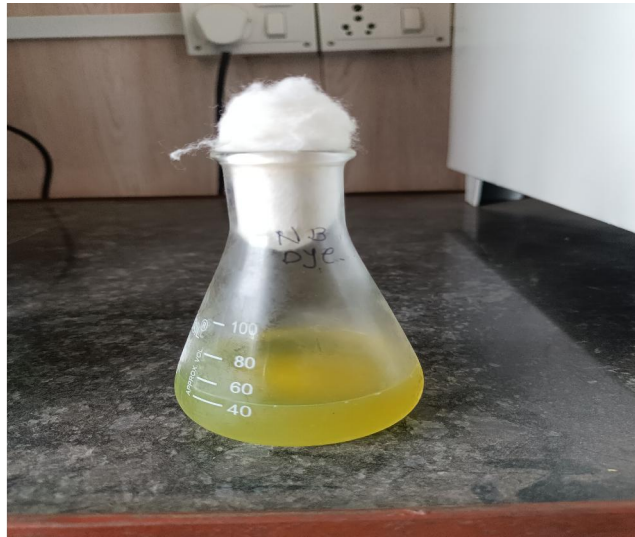
Gram staining of well isolated Colony











Biochemical characteristics

The result of biochemical characterization carried out are presented in table catalase test was the key for biochemical test done according to the key for identification of gram positive bacteria catalase is an enzyme that decompose hydrogen peroxide into oxygen and water molecules is introduced into hydrogen peroxide rapid calibration of bubbles of oxygen the gaseous product is produced

Citrate utilization test is a done a loopful of each culture was aseptically inoculated into the test tube containing koser's citrate medium. The media without inoculation was used to control the tubes were incubated for 24 hours at 37°C and checked for turbidity produce the test is positive



Character	Observation
Color	Orange
Shape	Rod
Margin	Entire
Elevation	Convex
Opacity	Non opaque
Consistency	Non sticky
Gram – staining	Gram – positive

Table - Morphological Characteristics of orange pigment

Character	Observation
Color	Green
Shape	Circular
Margin	Entire
Elevation	Convex
Opacity	Opaque
Consistency	Non – sticky
Gram – staining	Gram - negative

Table - Morphological characters of green, blue, brown, yellow pigment

Test	Observation
Catalase test	Positive
Indole test	Negative
Voges proskaur test	Positive
Methyl red test	Positive
Oxidase test	Positive
Gelatin test	Negative
Motility	Negative
Citrate utilization test	Negative

Table - Biochemical test of orange pigment

Test	Observation
Catalase test	Positive
Indole test	Negative
Voges proskaur test	Negative
Methyl red test	Negative
Citrate utilization test	Positive
Oxidase test	Positive
Gelatin hydrolysis test	Positive
Motility	Positive

Table - Biochemical test of green , blue, yellow, blue pigment

From the above observation orange pigment isolates was found to be Serratia species and green, blue, yellow, brown pigment isolates was found to be Pseudomonas Species



IV. SUMMARY AND CONCLUSION

- Total two isolates of dye producing bacteria were obtained from soil sample
- One isolate are gram positive rod and other one is gram negative circular
- One isolate produce orange colour pigment and other is green, blue , yellow, brown colour pigment are produced
- This is dye producing pigment are natural process it is used in textile industry
- The orange colour pigments producing bacteria are in Serratia species and green , blue, yellow, brown colour producing bacteria are in pseudomonas species
- The green, blue, yellow, brown dye extract was formed
- The blue coloured dye producing bacteria are toxic in nature the gain of toxicity use of antibiotics
- The green , brown , yellow coloured dye producing bacteria are non toxic in nature
- Dye binding agent are used as oxalic acid is a strong binding agent used in dye production

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