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ISOLATION OF PIGMENT-PRODUCING BACTERIA AND ITS ANTIBIOTIC POTENTIAL OF THE EXTRACTED PIGMENT

Nair Sreecha Chandran, C. Prabha Kumari,

Reshma K. Pillai and KS. Arya Prasad

Department of Biotechnology, CEPCI Laboratory and Research Institute, Kollam, Kerala, Thiruvananthapuram, India.

ABSTRACT

Daucuscarota (carrot) and *Solanumlycospersicum* (tomato) were collected from the local market of Kayamkulam and used in the study to extract pigments producing bacteria. From the observations the extracted bacteria were Gram Negative, cocci and biochemical test were done. The extracted pigment were checked for microbial activity towards it. The result was that the *Daucuscarota* (carrot) showed more antibiotic property towards *Listeria monocytogene, Pneumonia, S.typhii, P.aeroginosa* and *Solanumlycospersicum*(tomato) showed antibiotic property towards *Bacillus* and *Pneumonia.*

Keywords: Daucus*carota*, *Solanumlycospersicum,* Bacteria, Microbial activity and Dyeing.

1. INTRODUCTION

Dye is something which imparts colour to a substance such that the colouring is not easily degenerated by washing, heat, light, or other factors to which the material is likely to exposed. Dye, chemically binds to the substrate to which it is applied, this differentiates dyes from pigments which do not chemically bind to the material they colour. colour. Dye is usually prepared in aqueous solutions. (Booth et al. 2000). Dyes are coloured because they are absorbing visible lights. And dyes are soluble in water too. Natural dye are derived from plants, invertebrates, or minerals. The majority dyes are the vegetable dye from the sources like roots, barks, leaves etc and other biological sources such as fungi, algae, and lichens.

Use of natural dye than the synthetic dye is more effective. As we know natural dyes are derived from natural composition and it doesn't contain any chemicals. Natural dyes are not harmful to the environment as well as to human being. They are renewable resources. Some natural dyes have excellent fastness to light, cleaning agent, and perspiration. Natural dyes are principally perishable, eco-friendly and fewer allergic in nature. Synthetic dye which contain lots of chemical is harmful. Also it cause health hazards. So we could prefer natural dye which contain zero chemicals. This natural dye can used in food stuff. Natural dye can be obtained from two major sources plants and microorganisms. The advantages of dye production from microorganism include easy and fast growth in the cheap culture media. Microorganisms produce various pigments like carotenoid, melanins, flavones, quinines. The various types of microorganisms like bacteria, fungi, algae, yeast are present in different colors. The major objectives is to produce natural pigment from *Daucuscarota* (carrot) and *Solanumlycospersicum*(tomato).

2. MATERIALS AND METHODS

Collection of samples

Daucuscarota (carrot) and *Solanumlycospersicum (tomato)* samples were collected from the local market in Kayamkulam, Kerala, India.

Sample preparation

1gm of *Daucuscarota* (carrot) and *Solanumlycospersicum (tomato)* were mashed with 10 ml of distilled water and it was used.

Isolation and screening of pigmented microorganisms

The samples were serially diluted up to10^{-5} .1 ml of the diluted samples were spread on nutrient agar plate. Incubated for 24hrs at 30^{o}cand it was checked for the pigmented production. The pigment produced colonies were used for further studies.

Purification of culture

Pigmented Bacterial isolates was purified by streaking onto nutrient agar plate and it was incubated for 24hrs at 30^{0}c.

Maintenance of culture

Pigmented Bacterial cultures were grown on nutrient agar and it was maintained at 2-4^{o}c temperature in refrigerator and sub cultured into respective medium.

Characterization of isolated pigmented microorganisms

Gram stain

The stain makes use of the differing membrane structures between Gram positive (single cytomembrane with a troublesome outer plasma membrane of peptidoglycan), and Gram negative organisms (have 2layers of membranes, with a skinny layer of peptidoglycan sandwiched between them**.**

Prepare a bacterial smear and heat fixed on a slide, pour a few drops of crystal violet on a smear wait for 1 minute and wash with water. Now fixed the smear with Gram's iodine for 1 minute and wash again with water and decolourize the stain with 95% ethyl alcohol dropwise, wash with water and conter stain with saffranine (30 sec) and again wash with water. After drying examine under oil immersion.

Catalase Test

It is used to test for the presence of enzyme catalase. Hydrogen peroxide(H_2O_2) is made as associate outcome of the aerobic breakdown of sugars. When (H_2O_2) accumulates, it becomes toxic to the organism. Catalase decomposes (H_2O_2) and permits the organism to survive solely obligate anaerobes lack this protein. Bubbling (O_2 gas is liberated from the H_2O_2) shows positive result andno bubbling denotes negative result.

Glucose Fermentation Test

Add 0.1mg phenol red in sterile nutrient broth in test tube (6) where 3 as control. Add durham tubes in these test tubes containing 0.5% sucrose, 0.5% lactose, 0.5% glucose. Add 1 ml inoculator into this. Incubate at 37^{o}c for 24hrs. If there is an acid and gas formed, shows the positive result. If there is no acid and gas formation, shows negative result

Test for Antibiotic susceptibility

The performance of antimicrobial susceptibility testing is important to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates. The centrifuged pigments of *Daucuscarota* (carrot) and *Solanumlycospersicum* (tomato)were marked in the centre position of two separate agar plates and bacteria like*S.typhi, Bacillus, P.aeruginosa, Pneumonia, Listeria monocytogenes, E.coli* were inducedat both sides. Incubateedfor 24 hours at 37 degree celsius.

Extraction of pigments

There are different solvents such as ethyl acetate, methanol, acetone, hexane were used to check for the maximum solubility of pigments. And the solvent was selected by checking the maximum solubility of pigment in it. After incubation the bacterial cells were washed with methanol and it was transferred to centrifuge tube. It was centrifuged at 5000 rpm for 10 minutes. The colored supernatant was separated from the centrifuge tube into china dish. The supernatant is kept for water bath, and pigment is extracted.

RESULT AND DISCUSSION

1. Extraction of pigment

Pigments were extracted from the *Daucuscarota* (carrot) and *Solanumlycospersicum* (tomato).

Methanol was used for the pigment extraction and it is dried.

2. Antibiotic Test

The extracted pigment was tested for its growth inhibition towards bacteria. On nutrient agar plate the extracted pigment and bacteria (*S.typhi, Bacillus, P.aeruginosa, Pneumonia, Listeria monocytogenes, E.coli)* were induced. Incubated for 24hrs.*Daucuscarota* (carrot) showed inhibition towards *monocytogenes, Pneumonia, S.typhi ,P.aeruginosa .Solanumlycospersicum*(tomato) showed inhibition towards *Listeria monocytogenes,* Bacillus*.*

3. CONCLUSION

Natural pigment could be extracted from *Daucuscarota* (carrot) and *Solanumlycospersicum* (tomato) and could be

used to prevent the microbial growth. For bacterial inhibition, *Daucuscarota*(carrot) seemed much more effective than *Solanumlycospersicum* (tomato).

4. ACKNOWLEDGEMENT

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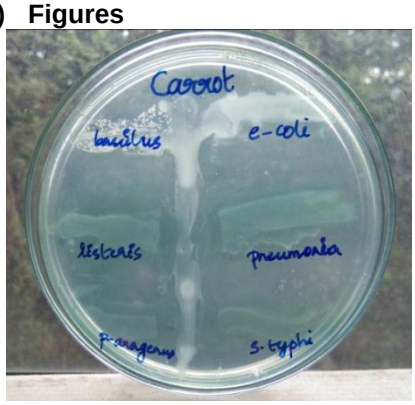
5. Tables and figures

a) Tables

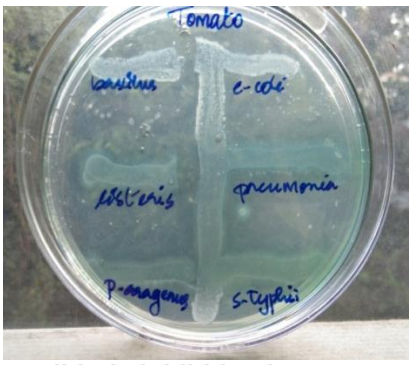
Table for biochemical Characterization of micro-organisms

Biochemical tests	Result
Catalase test	Negative
Glucose fermentation test	Negative

b) Figures



Antibiotic inhibition by carrot



Antibiotic inhibition by Tomato

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