Production of natural pigment from Beetroot extract

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ABSTRACT

Vegetable (Beetroot) sample collected from local market of Chinnakada near Kollam and and the different screening methods were performed for the identification of Microorganism. After the pigment isolation, extractions of the pigment were done. The extracted pigment was used to determine the antibiotic action and it can be used as a natural dye. In the present study Pseudomonas aeuroginosa were identified from the beetroot extract, extraction of pigment was done showing antibiotic susceptibility with the ability to be used as a natural dye.

Key Words: *Pseudomonas aeuroginosa, Bacillus cereus, Natural dye.*

1. INTRODUCTION:

Microorganisms is the organism which live in almost every habitat from the poles to the equator ,deserts, rocks and the deep sea. Some are adapted to extreme such as very hot or very cold conditions. They also make up the microbiota found in and on all multicellular organisms. Microorganisms are known as a potential source for bio pigment production due to their advantages over plants in terms of availability; stability; cost efficiency; labour; yield and downstream processing (1). Varieties of different biopigments have been produced such as carotenoids, melanins, flavins, quinines, monascins, violancein using microorganisms. Microorganisms such as Monascus, Rhodotorula, Bacillus, Achromobacter, Yarrowia produce a large number of pigments (2). A perfect pigment that produces microorganism should be capable of using a diverse range of carbon and nitrogen sources with tolerance to pH, temperature and minerals. Improvement in stability, safety, and solubility can help in making use of microbial pigments in the food cosmetics or textiles industry. A synthetic dye are widely available at a cheap price and produce a wide variety of colors but causes skin allergies and produces toxicity that shows chemical hazards during its synthesis (3), which releases undesirable hazardous toxic chemicals. Due to these hazardous effects of synthetic pigments, there is more market demand and custom interest towards natural or microbial pigments. Therefore the present study, extraction of pigment was done and antibiotic susceptibility were analysed.

2. METHODOLOGY

2.1 Collection of sample

Vegetable (beetroot) sample was collected from local market near Chinnakada Kollam, Kerala, India.

2.2 Isolation and purification of pigmented microorganism:

The sample was serially diluted up to 10 and 0.1 ml of the diluted sample was spread on nutrient agar plate. Incubated for 24 hours at 37°C and it was checked for pigment production .The pigment produced colonies were

used for further purification on nutrient agar and it was maintained at 2-4 degree celsius temperature in refrigerator and sub cultured into respective medium.

2.3 Characterisation of isolated pigmented microorganism and extraction:

Various biochemical test which was performed like gram staining, catalase test, Sugar hydrolysis test and different solvents like ethyl acetate, methanol, acetone hexane was used to check for the maximum solubility of pigments .In this study the bacterial cells of beetroot extract were washed with different solvents and were transferred to centrifuge tube .The tube was centrifuged at 5000 rpm for 15 minutes. The supernatant and pellet was separated. Then the supernatant is placed in the rotary evaporator and tested for antibiotic susceptibility.

2.4 Pigment dye test:

Pigment dye test was performed in the fabric cloth where the extracted pigment was applied to the cloth and kept for incubation for 72hr at room temperature.

3. DISCUSSION:

Natural pigments can be obtained from two important sources namely plants and microorganisms. The advantage of pigment production from microorganisms include easy and fast growing in the cheap culture media. The various types of microorganisms like bacteria, fungi and yeast are present in different colours. In the presence of pH the red pigment producing micro-organisms produce different coloured pigments where they changed the temperature, and at above 60^{o}C, it shows a colour change from red to pink (4). They concluded that the micro-organism responsible for pigment production *Rhodotrula species,* when we done this we came into the conclusion that the microorganism responsible is *Staphylococcus aureus*.

In a study, author reported that when the Red pigment producing colonies are treated with different pH, different colour pigments are produced which shows the various colours produced when pH & temperature was changed (4). But in our study a comparative study of beetroot pigments with other two organism pigments such as *Pseudomonas aeruginosa* and *Bacillus cereus* was done. Further pigment extraction were done from the same followed by centrifugation and evaporation getting green coloured pigment (*P. aeruginosa* - green).

Further studies were done to determine whether the extracted pigment shows dyeing property After staining the fabric cloth using different samples and then keep it for 3 days .When washed it in tap water others will washed out and the *Pseudomonas aeruginosa* remain in the fabric cloth as a green coloured pigment. It proves that *Pseudomonas aeruginosa* can be used as pigment dye in fabric industries.

4. RESULT:

4.1 Screening

The bacterial colony from serial dilution was screened by quadrant streaking and pure cultures were obtained. Further Gram staining, Biochemical and morphological characteristics was done for identifying the morphology (Table 1) and nature of the bacteria which was found out to be *Pseudomonas aerugonisa* (Figure 1).

4.2 Antibiotic susceptibility test:

Six species which were pathogenic were taken for the test such as *listeria, S. typhi, S. aureus, K . pneumonia, Z. mobiles, P. floursen. Pseudomonas aerugonisa* showed inhibition activity against three species such as *S. aureus, K pneumonia, Z mobiles* (Figure 2).

4.3 Pigment dye test

Pigment dye test was performed in the fabric cloth where the extracted pigment was applied to the cloth and kept for incubation for 72hr at room temperature. After the incubation, it showed that stain on Pseudomonas aeruginosa stayed in the cloth (Figure 3) which indicated high potential of natural dying property.

5. LEGENDS:

5.1 Tables

Table 1- Biochemical and morphological characteristics

Tests	Result
Gram‘s Staining	Gram Negative
Shape	Rod
Catalase	Positive
Indole Test	Negative
Citrate Utilization Test	Positive
Mannitol fermentation test	Positive
Oxidase Test	Positive

5.2 Figures

Figure 1- Pure Culture of Isolate

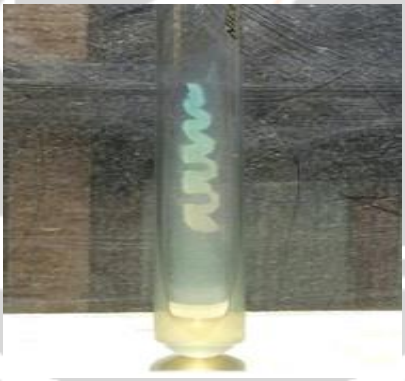
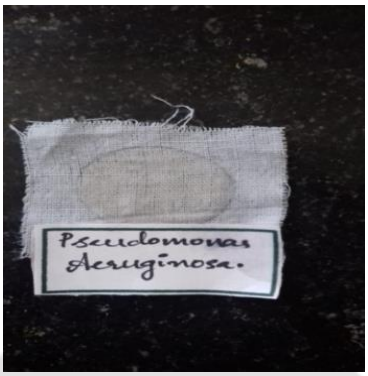


Figure 2- Antibiotic susceptibility test for *Pseudomonas aeuroginosa*

Figure 3- Pigment dye test



6. CONCLUSION:

The *pseudomonas aerugonisa* showed highest antibiotic inhibition against *S. aureus, K pneumonia, Z mobiles* and during pigment dye test, the pigment remain in the fabric cloth as a green colored pigment. It proves that *pseudomonas aeuroginosa* can be used as pigment dye in fabric industries with high potential of natural dyeing property.

7. ACKNOWLEDGEMENT

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8. REFERENCES:

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