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Isolation of *Serratia marcescens* and evaluation of its Congo red degradation activity

Aditya Kashikar* and **Kishori Tarfe**

Department of Biotechnology, Smt. Chandibai Himathmal Mansukhani College, Ulhasnagar, District Thane, Maharashtra, India

*Corresponding author E-mail:- adityakashikar96@gmail.com

Pigments are natural as well as manmade biochemical compounds produced by plants and microorganisms which exhibits specific colors. They are regularly utilized in dye & textile industries, cosmetics, medicines, etc,. and also posses specific beneficial abilities along with antimicrobial activity. Prodigiosin pigment is one of the widely known and studied pigment which is produced mainly by *Serratia marcescens* It possess abilities like cellulose production ,dye degradation and antibacterial activity. It also shows different colors on different media. Among the various sources taken, the pinkish growth on coconut (*Cocos nucifera*) was collected. Red pigmented colonies on plate indicated presence of *Serratia marcescens*, primarily confirmed by Gram staining when Gram negative coccobacilli observed and later confirmed by biochemical tests. Extraction process for pigment was carried out by swabbing-overnight methanol method. The absorption spectrum of extracted pigment was observed on Digital colorimeter and. Plates swabbed with culture in peptone water resulted in pink coloured growth at room temperature. This particular research article deals with the Congo red degradation ability analysis of *Serratia marcescens* by using Broth inoculation and Colorimetric method. It has wide applications in colorants, dyes, textile industries, medicines, cosmetics. The dyeing activity of extracted pigments can be checked using industrial binders and can be used if found environmentally harmless. Anti-microbial activity of pigment is one of the strong aspect of Prodigiosin and can be checked using AST techniques such as disc-diffusion or Agar-cup method against different pathogens.

Key words :- *Serratia marcescens*, Prodigiosin, Azo dyes, Dye Decolorization Assay, Congo red

INTRODUCTION

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. Nature is rich in colors obtained from fruits, vegetables, roots, minerals, plants, microalgae, and so forth[1]. Pigments are compounds with characteristics of importance to many industries. In the food industry they are used as additives, colour intensifiers, antioxidants etc. Various pigments are produced by microorganisms which vary in colour from red, yellow, orange, purple etc [2]

Nature produces many biocolorants from various resources including plants and microbes which are possible alternatives to synthetic dyes and pigments used nowadays. Natural pigments are sourced from ores, insects, plants and microbes. Biopigments produced from microorganisms are widely used than of plants due to their readily availability and wide varieties [3]

Many synthetic colors used in foodstuff, dyestuff, cosmetics and pharmaceutical manufacturing pose various hazardous effects like allergies, tumor, cancer and severe damages to the vital organs. Consequently, many synthetic colors have been banned due to their toxicological problems. With the increasing awareness about the toxic effects of synthetic colors and consumer safety, there is an increasing interest in the development of colors from natural sources [4]

Natural colors are generally extracted from fruits, vegetables, roots and microorganisms whose eco-friendly, antioxidant, anticancer and antimicrobial activities further add to their positive effects. The significant growth in the naturally derived colors has been attributed to their stability and consumer acceptance.. Although there are a number of natural pigments, only a few are available in sufficient quantities to be useful for industry because they are usually extracted from plants [2]

Bacteria are 2nd biggest source of natural pigments after plants. Each pigmented bacteria possess unique colour or different shades of colour. Apart from the coloring , the pigments show various activities in nature, mostly favorable for other living organisms. Some of the activities were detected or evaluated in this project. Among the various pigmented bacteria present in nature, *Serratia marcescens* and *Sarcina spp.* are the notably evaluated in world and are well known for their various activities. These organisms produce some of the important pigments in nature- Prodigiosin and Carotenes respectively Pigments

are different than dyes due to their difference between solubility.dye gets dissolved in water while pigment forms precipitation. Therefore they are dissolved in solvents like Methanol and Petroleum ether [5]

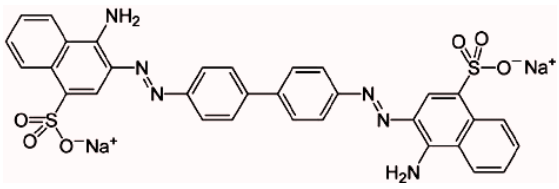
Many pigmented microbes posses various activities such degradation of harmful substances such as dyes, reagents, radioactive materials, cellulose etc,. [7][8] They also have anti-cancerous, antioxidant, anti-malarial properties[6]. Many bacterial pigments have wide range of antibacterial activity against pathogens such as *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Bacillus subtilis, Klebsiella pneumonia, Shigela sonnei, Pseudomonas aeruginosa* etc,.[2] . Disc-diffusion method can be used for AST. Prodigiosin pigment produced by *Serratia marcescens* is said to have anti-malarial property[6]

Pigment changes the colour of incident light. Pigments such as *Serratia marcescens* have wide range of applications beneficial to nature such as degradation of Congo red, production of Cellulase enzyme to degrade cellulose, antibacterial activity against many pathogens etc. [2]

Azo dyes

Azo compounds are organic compounds containing one or more azo bonds (N=N-) bound to aromatic ring. Most of the azo compounds get reduced to common aromatic amine, arylamine by Reductive cleavage or enzymatic reactions in body and sometimes due to exposure to light and high temperature. They are widely used in textile fibers such as cotton, silk, wool, viscose and synthetic fibers. Arylamines are allergic to skin, eyes and toxic if swallowed or inhaled. They also cause long term adverse effects in aquatic environment [9]

One of the widely used azo dye in textile industries is **3,3'-(Biphenyl- 4,4'-diyldidiazene -2,1-diyl) bis (4-aminonaphthalene-1-sulfonic acid)** known as **Congo Red**. Sometimes it is also recognized by other names such as Kongorot, Cosmos Red, Cotton Red B, Cotton Red C. It is odorless, carcinogenic secondary diazo compound generally used in production of inks, paints, indicators and reagents[10]



Chemical strcture of Congo red ($C_{32}H_{24}N_6O_6S_2$) [11]

MATERIALS AND METHODS

Sample collection :-

Serratia marcescens mainly grows on coconut fruit. Hence the white meat part (copra) of Coconut fruit (*Cocos nucifera*) was collected and kept in moist environment in laboratory refrigerator of Dept. of Biotechnology, Smt. C.H.M. College, Ulhasnagar, Maharashtra, India for 1 week. Pink growth was collected as sample for further identification and testing.

Methods

(A) **Isolation**[2] **:-** Nutrient broth (HiMedia Laboratories) was used for isolation. The pinkish growth on coconut fruit was mixed in St. Saline and streaked on St. Nutrient Agar plate using Penta-streak method.

(B)**Identification**[12][13]**:-** Isolated red colonies were subjected for identification using Glucose-phosphate broth , Simmon Citrate agar , Tryptone broth (IMViC test) and Triple sugar iron agar (TSI test). Also Gram nature was determined using Gram's staining reagents

(C) **Enrichment**[14] **:-** Three flasks of 100ml Nutrient broth each containing different concentration of Congo red dye (1%, 0.01%. 0.005%) were prepared and autoclaved. 5ml of bacterial suspension was aseptically inoculated in broths. Flasks were kept for incubation at room temperature.

(D) **Dye Decolorization Assay**[14] **:-** As organism gradually degrades the dye or reduce it to harmless byproducts, the colour intensity of dye decreases. After definite intervals of days, 3-4ml of inoculated broth from each flask was pipette out aseptically in St. centrifuge tubes and centrifuged at 2500 rpm for 15 min and then supernatant was used to determine optical density measured colorimetrically at 465nm wavelength.

(E) **Percentage of Dye-Decolorization**[14] **:-** It can be calculated by following formula

$$\text{Percentage of Dye Decolorization} = \frac{\text{Initial O.D.} - \text{Final O.D.}}{\text{Initial O.D.}} \times 100$$

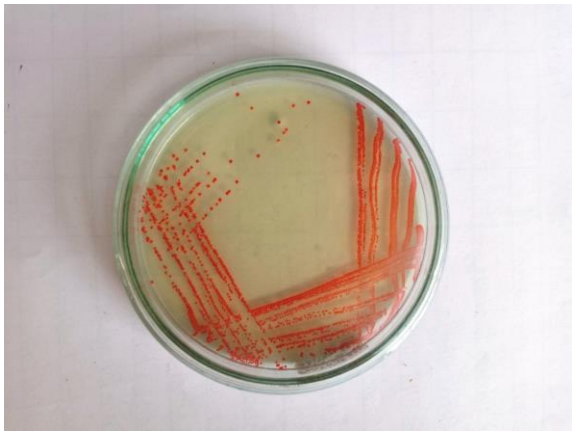


Fig. 1 : Red colored *Serratia marcescens* isolated from coconut fruit under moist conditions using Penta-streak method

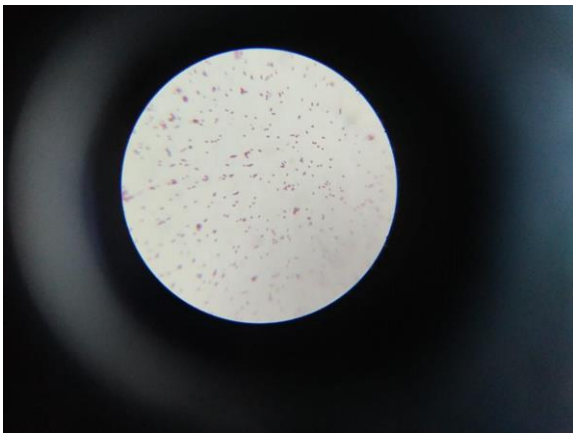


Fig. 2 : Pink coloured Gram negative coccobacilli (*Serratia marcescens*) observed after Gram's staining under oil immersion lens (1000X)

Fig. 3 : Conical flaks containing 100ml St. Nutrient broth in each flask and with different conc. of Congo red (1%, 0.01%, 0.005%)

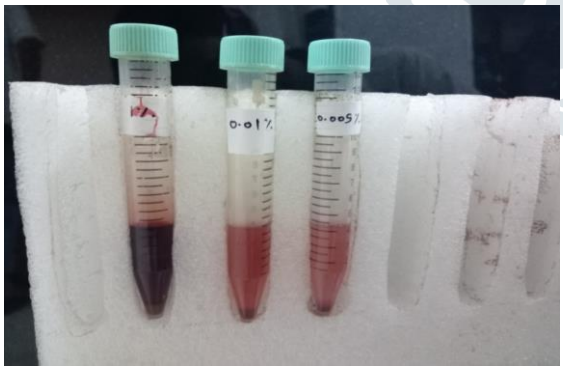


Fig. 4 : Centrifuge tubes with 1%, 0.01%, 0.005%, Congo red+ NB on Day 1

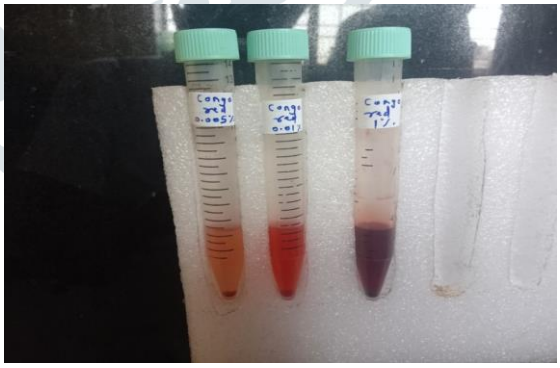


Fig. 5 : Centrifuge tubes with 0.005%, 0.01% and 1% Congo red+ NB on Day 10

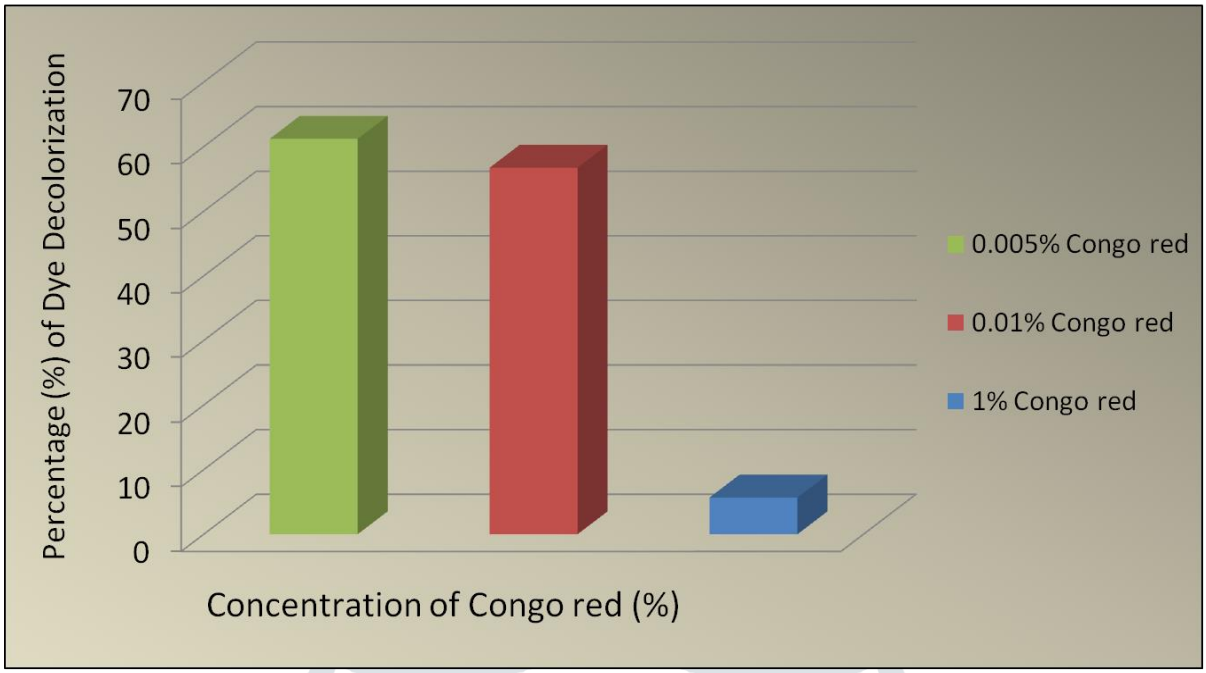


Chart 1 : Graphical Representation of Dye Decolorization assay of Congo red of conc. 0.005%, 0.01%, 1% at absorption wavelength 465nm

OBSERVATIONS AND RESULTS

Absorption wavelength (nm) (Digital Colorimeter)	Optical Density (O.D.)		
	NB + Congo red (1%)	NB + Congo red (0.01%)	NB + Congo red (0.005%)
425	1.80	1.37	0.44
465	**3.34**	**2.52**	**0.49**
490	2.54	1.86	0.43
515	2.42	1.81	0.41
525	1.57	0.69	0.21
545	2.85	1.08	0.27
570	2.24	0.50	0.13
615	1.05	0.05	0.02
650	0.72	0.05	0.03

Table 1 : Absorption spectrum of Nutrient broth containing Congo red (1%, 0.01%, 0.005%) measured on Day 0 using Digital Colorimeter

The maximum absorption for all three mediums were found at **465nm**

Colorimetric data of Dye - Decolorization Assay:-

Day	Absorption wavelength (nm)	Optical density		
		NB + Congo red 1%	NB + Congo red 0.01%	NB + Congo red 0.005%
0	465 nm	3.34	2.52	0.49
1		3.26	2.06	0.43
3		3.22	1.70	0.39
7		3.19	1.40	0.26
10		3.15	1.09	0.19

Table 2 : Optical density of all three mediums at 465nm on definite intervals of days

Calculations :-

Concentration of Congo red in Nutrient Medium	1%	0.01%	0.005%
Percentage (%) of Dye Decolorization	5.68 %	56.74%	61.22%

Table 3 : Percentage dye-degradation (calculated by Std. formula)[14]

DISCUSSION

Water pollution is one of the critical and health disturbing problem in environment. Despite regulations and safety measures for releasing wastewater in water bodies, many industries carelessly release the waste water containing hazardous chemicals, dyes, waste byproducts into water bodies which is harmful to aquatic life and surrounding ecosystem which justifies need of more conventional eco-friendly filtration techniques are needed.

To explore the microbial bioremediation The conducted study included isolation of pinkish growth on coconut which was confirmed later to be Gram negative coccobacilli. The isolated organism responsible for production of red pigment and decolorization of Congo red was confirmed to be *Serratia marcescens.*

The percentage of dye decolorization was calculated using formula illustrated by K.R Mahbub, A Mohammad, A.A ahmed, Salma Begum[14] . The percentage decolorization was found to be 61.22%, 56.74% and 5.68% for Congo red of concentrations 0.005%, 0.01% and 1% respectively. Degradation of Congo red dye at 1% concentrations started late as compared to 0.005% and 0.01% concentration. As O.D. of 1% was obtained > 1.0 still there was decrease in color intensity observed. Conducted assay also revealed that the colour intensity & O.D was constant throughout the incubation period (Day 0 to 10). This indicates that dye degradation is not according to the incubation period, but due to presence and increase of number of microorganisms in the medium.

In a advanced study of Congo red degradation, conducted by Jian Sun, Youming Li, Yongyou Hu, Bin Hou, Yaping Zhang & Sizhe Li, the mechanism of Congo red degradation and bacterial diversity in a single-chambered microbial fuel cell (MFC) incorporating a microfiltration membrane and air–cathode was planned and observed. The study included MFC with Congo red and Glucose as fuel. Congo red azo bonds were reduced at the anode to form aromatic amines indicating the anaerobic biodegradation of azo dyes. Less dense

biofilm was observed at the anode in presence of Congo red compared to it absence indicating that Congo red negatively affected biofilm formation.[15]

In a study conducted by Nedra Asses, Lamia Ayed, Neila Hkiri, and Moktar Hamdi , fungal species *Aspergillus niger* was used as biodegrading agent against Congo red. Effect of various factors on decolorization and enzyme production was observed by *A. niger* which plays essential role in decolorization and detoxification such as lignin peroxidase and manganese peroxidase. Decolorization percentage increased from 45% to 98% in six days of incubation confirming that shaking helps in mixing of oxygen in medium allowing more Aspergillus to grow and decolorize the dye. The researchers concluded that 1g of fresh biomass can eliminate 27% of CR dye by adsorption mechanism and 70% by enzymatic biodegradation.[16]

CONCLUSION

Isolation of dye degrading bacteria was done using Nutrient agar and white fleshy part (copra) of coconut fruit as a sample. The organism was later identified as *Serratia marcescens* using IMViC (--/+/+) and TSI Lactose(-)Sucrose(+)Glucose(+).The organism isolated was preserved and utilized for Dye-decolorization for evaluation of its dye-degrading strength. Three different media with Congo red concentration of 1%, 0.01%, and 0.005% were prepared and subjected for inoculation and evaluation. The % dye decolorization activity for 1%, 0.01% and 0.005% were found to be 5.68%, 56.74% and 61.22% respectively. Isolated organism can play essential role in degradation of pollutants. Mostof the times, organisms like this reduce the dye or harmful chemical structurally to harmless by-products. Biological control products involving decolorizing property of *S.marcescens* and similar organisms can be employed into Sewage water treatment and industrial outlet filtration. Moreover prodigiosin pigment from *Serratia marcescens* is a therapeutic agent for prevention and cure of diabetes mellitus without any side effect.It also posses anti-cancerous, anti-malerial and immunosuppressive activity which can be explored further.

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