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Studies on Extraction and Applications of Prodigiosin from Serratia Marcescens

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Abstract-- Prodigiosins, a natural red pigments is categorized by a common pyrrolylpyrromethane skeleton.It is produced by *Serratia marcescens.* This pigment possesses the characteristics of anti-fungal, anti-cancer and anti-proliferative activity. This prodigiosin pigment is a secondary metabolite produced by Gram -ve and Gram +ve bacteria. The prodigiosin is produced by several bacteria. It was first characterized from *Serratia marcescens*. The *Serratia marcescens* produces red pigment, which was tested for presence of prodigiosin by presumptive colour test. Its adsorption spectra and quantification is carried out on nutrient agar medium. In the acidified solution a pink colour and in the alkaline solution yellow colour indicate positive presumptive test for prodigiosin pigment. In case of adsorption spectra; using spectrophotometer in the range between 300 to 700nm the ethanolic acidified and alkalinized red pigment extract was tested. For the ethanolic acidified red pigment extract the maximum absorbance was at 535nm while for ethanolic alkalinized red pigment extract the absorbance was at 470nm.

Keywords- prodigiosin; Serratia marcescens; pigment

1. INTRODUCTION

Serratia species are categorized in the huge family of Enterobacteriaceae. They are gram negative bacteria. *Serratia* species can be differentiated from other classes by its capacity of production of three enzymes DNSase, Lipase and gelatinase. It includes the enzyme activities like nuclease, protease and haemolysin. *Serratia marcescens* are found in water, soil, man and on plants, animals. *Serratia marcescens* was used since 1900 by physicians to study spread of microorganisms (Anita Khanafari *et al*., 2006).

Prodigiosin has the capacity of inducing the apoptosis in the broad range of cancer cell lines, its pro-apoptotic effect is selective against malignant cells. Prodigiosin is also found to be a promising anti-cancer agent .Action Mechanism of prodigiosin is important for the development of drug and characterization of the still unidentified cell target.

The prodigiosin belongs to the tripyrrole red pigment family which contains a 4-methoxy, 2-2 bipyrrole ring system. The synthesis of prodigiosin is a diverged procedure in which mono and bipyrrole precursors are synthesised discretely. Then they are assembled to form prodigiosin pigment which has been related to extracellular vesicles. (Doona *et al*, 2014).

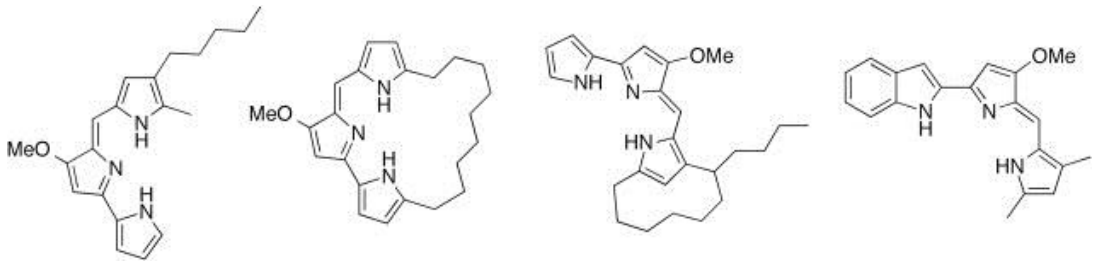


Fig 1. Structure of prodigiosin (Doona *et al*. 2014)

TABLE. 1 LIST OF SIGNIFICANT NATURAL PIGMENTS AND ITS APPLICATIONS

	Producing organisms	**Pigment**	**Colour**	**Application**	**Reference**
1	Filamentous fungi *Candida famata,* and bacterium	Riboflavin	Yellow	Baby foods, fruit drinks, milk products,	Stahmann *et al.*

	Bacillus subtilis			enriched with vitamin and energy drinks.	2000
2	*Phycomyces Mucor circinelloides. Blakeslea trispora*	Beta Carotene	Red, orange and yellow	Colourants in food	Malik *et al.* 2012
3	*Myxococcus Mycobacterium*, *Agrobacterium Sulfolobus Serratia* and *Streptomyces.*	Carotenoids	Orange red	Food colourant	Browning *et al.* 2003 Yokoyama *et al.* 1994
4	*Serratia marcescens, Vibrio psychoerythrus, Streptoverticillium rubrireticuli* and other eubacteria	Prodigiosin	red	Anti-malarial and Anti-bacteria	Khanafari *et al.* 2006
5	*Cyanobacteria, spirulina.*	Phycocyanin	Blue	Dietary supplement	Malik *et al* 2012

(Referred from N Darshan and H K Manomani, 2015)

2. METHODOLOGY

A. Isolation of Serratia marcescens

Serratia marcescens was obtained from the soil and water sample. By plating 0.1ml of soil suspension over the surface of nutrient agar plate, in 10^{-6} dilution approximately three colonies of *Serratia marcescens were observed* from 50 colonies. The frequency of the prodigiosin producing strain was found to be 3.0×10^{-7} CFU m L^{-1} and 3.0×10^{-9} CFU $g^{-1.}$ Thus, through the routine laboratory method the species was identified (Patel, 2011).

Serratia marcescens was cultured in nutrient broth with varying glycerol concentrations incubated at 25˚C at 150 rpm for 72 hours to acquire a higher production of prodigiosin. After incubation of 72 hours, microorganisms were able to produce soluble prodigiosin which was confirmed by visualizing the broth as well as cultural characteristics on nutrient agar plate. Gram reaction was found to be negative which confirmed one of the key characteristics of *Serratia marcescens* (Boone and Castenholz, 2001).The extraction of pigment was carried out (Gunasekaran, 2005) and re-dissolved in methanol and ethyl acetate.

B. Presumption test and purification of extracted pigment

Presumption test was accomplished for the confirmation of prodigiosin pigment (Satish kumar and Aparna, 2014). Using different solvents such as 95% methanol, chloroform, ethanol and ethyl acetate the centrifugation of broth was achieved. The pellet was discarded and supernatant was tested against alkaline and acidic conditions. Pink colour in acidic condition and yellow colour in alkaline condition confirmed a positive presumptive test for red pigment prodigiosin.

The bacterial cell culture showed absorbance at 620 nm. For the pigment absorbance, the broth was centrifuged using methanol and supernatant was taken for measurement of absorbance at 534 nm. The following formula is used for estimation of prodigiosin.

Prodigiosin (unit per cell) = $[OD_{534} - (1.381\times OD_{620})] \times 100$

(1)

OD_{620}

Where, OD_{534}= Absorbance of pigment

OD_{620}= Absorbance of bacterial cell culture

1.381= Constant

Purification of prodigiosin pigment can be carried out using thin layer chromatographic technique (TLC).The separation of pigment using TLC plate which is coated with silica gel. The developing solvent such as chloroform**,** methanol and acetone (4:2:4 v/v) is used in the chromatographic tank. The solvent was standardized and powdered, than it was saturated with the filter paper soaking in a mobile phase. Than the RF (retention factor) value was calculated (shrimathi*et al*. 2017).

3. APPLICATIONS STUDIES OF PRODIGIOSIN

A. Anti-bacterial activity of pigment

The anti-bacterial activity of prodigiosin was studied on nutrient agar disc diffusion technique which was carried against isolates of gram-negative bacteria. Filter paper discs were soaked with 20 µl of methanolic pigment. Here 95% of methanol was taken as negative control and streptomycin was taken as positive control. The dried discs were placed on the test bacterial surface and incubated for 18-24 hours at 37°C. The zones of inhibition were formed around the discs showing the anti-bacterial activity (Vora *et al*. 2002).

B. Total anti-oxidant capacity

The phosphomolybdenum method was used to estimate the anti-oxidant capacity of pigment (Prieto et al., 1999).1ml of reagent solution (28mM sodium phosphate, 4mM ammonium molybdate and 6M sulphuric acid) was mixed with 0.1 ml of extract solution. The reaction was incubated for 90mins at 95°C.Using spectrophotometric method the absorbance was measured at 695nm. The anti-oxidant property was measured as equivalents of ascorbic acid.

C. Effectiveness as dyeing agent

In different test tubes the fabrics such as cotton of size $3cm^2$ were soaked in methanolic extract of prodigiosin pigment. The test tubes were incubated at 48 hours at room temperature and then dried. The pieces were treated with alkali, acid, cold water and detergent. Then in hot water and detergent for 1 hour in test tube respectively (Chandni Gulani *et al.,* 2012).

4. RESULTS AND DISCUSSIONS

Prodigoisin pigments produced by *serratia marccescens* possess huge efficiency as a dye and as medically important products. Prodigiosin exhibits anti-oxidant, anti-microbial properties. Prodigiosin can also be used as red dye in textile industry. The recent investigation focused on formulation of an extraction protocol for prodigiosin production and its applications.

A. Isolation of Serratia marcescens and extraction of pigment

The nutrient agar plates were streaked with serially diluted soil samples and incubated at 30˚C for 72 hours. The red colonies observed on the plates which indicated the presence of *Serratia marcescens (fig 2).*

The colonies obtained were sub cultured in 100ml nutrient broth and incubated at 30˚C for 72 hours at 150 rpm. The color of broth was changed to red color due to production of prodigiosin pigment (fig 3).

The solvent like methanol was added to culture broth containing pigment was centrifuged at 8000rpm for 10minutes and it was observed that the pigment was present in the supernatant (fig 4).

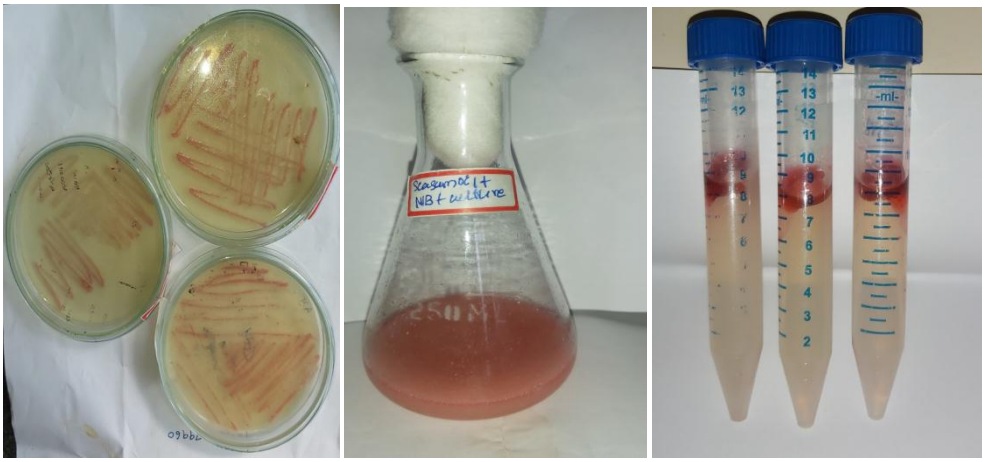


Fig 2 *S.marcescens* Fig 3.Production of Prodigiosin Fig 4.Extraction of prodigiosin

B. Presumptive test for prodigiosin pigment

The supernatant produced pink color when added with acid where as it showed yellow color when added with alkali, through this test it was confirmed that the red pigment produced was prodigiosin (fig 4)

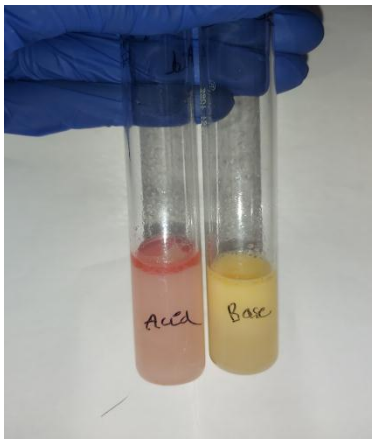
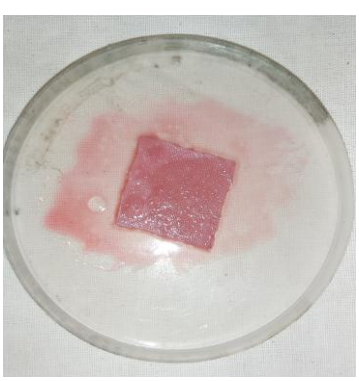


Fig. 5 Acid base test for confirmation of prodigiosin

C. Effectiveness as dyeing agent

The cotton fabric of $3cm^2$ was dyed with extracted prodigiosin pigment and was left for 48 hours and then washed in cold water with detergent and then the cotton fabric was air dried. The cloth was able to take the dye at a period of 2 hours and was able to retain the dye after exposing it to washing with water and detergents .The retention of pigment observed was found to be very effective.



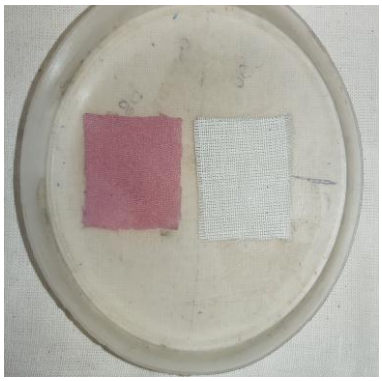


Fig 5 Dyeing of cotton fabric Fig 6. Comparison between dyed cotton fabric with control

CONCULSION

The recent investigation reveals that prodigiosin pigment produced by *Serratia marsecens* isolated from soil and water samples has clinical and industrial applications. The study deals with successful production and extraction of red pigment, prodigiosin produced by *S.marcescens.* The prodigiosin pigment has the potential to act as antimicrobial, anti-oxidative agent and also has good dyeing capacity. The prodigiosin obtained is economically effective and considered as natural product for further pharmaceutical applications. As prodigiosin pigment is produced naturally(microbial source) it is considered to be biodegradable and the process is eco friendly and better than synthetically produced dyes.

Reference

- Anita Khanafari,et al, "Review of prodigiosin ,pigmentation in Serratia marcescens",Online Journal of Biological science,vol.6(1), pp.1-13,2006.
- N Darshan and H K Manonmani, "Prodigiosin and its potential application",Journal of food science and technology, vol 52(9), pp.5389-5407,2015.
- M Donna,et al, "Natural product chemistry and cancer drug discovery" ,Cancer drug design and discovery, (2), pp.91-120,2014.
- Mansi Mehta and Gaurav Shah, "Extraction of pigment from Serratia marcescens and its application in candle industry", Indian Journals, vol 7(2), pp.1-4,2015
- M Patel, et al, "Identification of residue in chromodomain helicase DNA-Binding protein 1(Chd1) required for coupling ATP hydrolysis to Nucleosome Sliding" Journal of Biology and chemistry, vol 286(51), pp.43984-43993, 2011
- Boone and Castenholz, "The archaea and the deeply branching and phototropic bacteria", Bergeys Manual of systematic Bacteriology, vol 1, pp.1-721,2001
- Dr.nagesh Malik and Prachi Bhatia, "Use of prodigiosin pigment from Serratia marcescens as a dye for textile industry and highlighter pen Ink"
- R Srimathi, R Priya, M Nirmala and M Malarvizhi, "Isolation, Identification, Optimization of Prodigiosin pigment produced by Serratia marcescens and its Applications", International journal of latest Engineering and Management Research, Vol 2, pp.11-21, 2017.
- T Satish Kumar and H Aparna, "Anti-biofouling activity of prodigiosin, a pigment extracted from Serratiamarcscens, "International journal of current Microbiology and Applied Science, Vol 3, pp. 712-725, 2014.
- J H Park and C Itakura, "Detection of rabbit haemorrhagic disease virus antigen in tissue by immunohistochemistry", Research in veterinary science, Vol 52, issue 3, pp.299-306, 1992.