Research Article

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SCREENING OF *SERRATIA MARCESCENS* ISOLATED FROM SOIL FOR SECONDARY METABOLITES OF THERAPEUTIC IMPORTANCE

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Abstract: Microorganisms are known to produce pigments. The pigments produced by bacteria have received the attention of many investigators. Soil isolates obtained in our campus produced a pink to red color pigment when grown on nutrient agar. This isolate was identified as *Serratia marcescens* by MALDI – TOF mass spectrophotometer. The pigment was extracted and identified to be Prodigiosin. GC-MS analysis of the methanol fraction of the culture filtrate of *Serratia* sp reveals the production of various alkaloids of therapeutic importance. To the best of our knowledge this is the first report on the production of these compounds by *Serratia marcescens.*

Keywords: *Serratia marcescens*, Maldi-TOF, Prodigiosin, Therapeutic Products.

INTRODUCTION

Biopigments can be obtained from two major sources, plants[1] and microorganisms [2].Biopigments from the microorganisms have been preferred over those from plants because of their rapid growth and their availability throughout the year because of the cultivation technology[3,4]. *Serratia is* a Gram negative bacterium and belongs to Enterobacteriacea family. *Serratia marcescens* was differentiated from other enteric bacteria due to its characteristic red pigmentation. However many species of *Serratia* are non-pigmented or vary widely in pigmentation. Other well-known species include *S. odorifera, S. liquifaciens, S. rubidaea, S. ficaria, S. pymuthica* and *S. fonticola. Serratia marcescens* produces a cell-associated pigment called prodigiosin[5], which gives it the appearance of tiny blood red droplets on suitable growth media. Factors such as medium composition and oxygen supply, affect the production of prodigiosin and the incubation at 37^{0}C inhibit the pigmentation. Many types of differential and selective media have been developed for the isolation and presumptive testing of *Serratia*. It occurs in water and soil, on plant, in insects and in man and animal[6].

Prodigiosin is found to act against pathogenic bacteria, fungi, and parasite. It is a good immunosuppressive and anticancer agent. It is produced by *S. marcescens, Pseudomonas magneslorubra, Vibrio psychroerythrous etc.*[7] The prodigiosin group of natural products is a family of tripyrrole red pigments that contain a common 4-methoxy, 2-2 bipyrrole ring system. The biosynthesis of the pigment is a bifurcated process in which mono and bipyrrole precursors are synthesized separately and then assembled to form prodigiosin[8].

MATERIALS AND METHODS

Isolation and identification of *Serratia sp.*

Soil samples were obtained from the garden of CMS college, Coimbatore. The sample was subjected to serial dilution and plating on nutrient agar at 30° C to screen for the pigment producing strains. The red pigment producing isolate thus obtained was then subjected to morphological, biochemical and MALDI-TOF analysis for identifying the organism.

Production of pigment in nutrient broth:

The nutrient broth was prepared and inoculated with the isolated strain and incubated at 30°C in a orbital shaker at 210 RPM for 24 hrs.

Pigment extraction:

The nutrient broth inoculated with the isolated organism was observed for the pigment production. The culture broth was centrifuged at 10,000 rpm for 10mts. The supernatant was discarded and the pellet was suspended in 95% methanol to extract the pigment from the cells. The suspended pellet was centrifuged at 10,000 rpm for 10mts. Debris was removed and the supernatant was taken in two test tubes. The content of one of the test tube was acidified with a drop of concentrated hydrochloric acid and the other was alkalinized with a drop of concentrated ammonia solution. A red or pink color in the acidified solution and a yellow or tan color in the alkaline solution indicated a positive, presumptive test for prodigiosin[9].

Estimation of Prodigiosin

Isolated prodigiosin was estimated using the following equation.[10]

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$$\text{Prodigiosin unit/cell} = \frac{[OD499 - (1.381 \times OD620)] \times 1000}{OD\ 620}$$

OD 499 – pigment absorbance
OD620 – bacterial cell absorbance
1.381 – constant

Table.1: List of therapeutically important secondary metabolite produced by *S. marcescens*

S.no	Name of the compound	Molecular formula	Molecular weight	Retention time	Therapeutic Importance
1)	Imidazole [1,2-c]quinazoline (cas)	C10H7N3	169	3.22	Inhibition of ADP-induced platelet aggregation [18]
2)	Daurisoline	C37H42N2O6	610	20.07	bis-benzylisoquinoline alkaloid – The antiarrhythmic effect [19]
3)	Temuconine	C37H42N2O6	610`	20.07	Antiplasmodial and cytotoxic activity - bisbenzylisoquinoline alkaloids [20]
4)	8-(Ethoxycarbonylmethyl) caffeine	C12H16N4O4	280	26.92	Anticancer [21]
5)	Koumidine	C19H22N2O	294	34.82	Cinchonine drug – Chinese medicine [22]
6)	Corydione	C20H15NO6	365	36.78	1)Lower blood sugar levels 2) Decreasing fat storage 3) Antibacterial and anti-inflammatory effects 4) Potential anticancer (anticarcinogenic) activities: Inhibit the growth of SC (skin cancer) cell [23]
7)	Loperamide	C29H33ClN2O2	476	38.71	Anti diarroheal [24]

GC-MS Analysis: GC–MS analysis was performed with reversed-phase (RP) chromatography by using a solvent system of methanol and the carrier gas He with flow 1.0ml/min. This analysis is carried out with THERMO MS DSQ II.

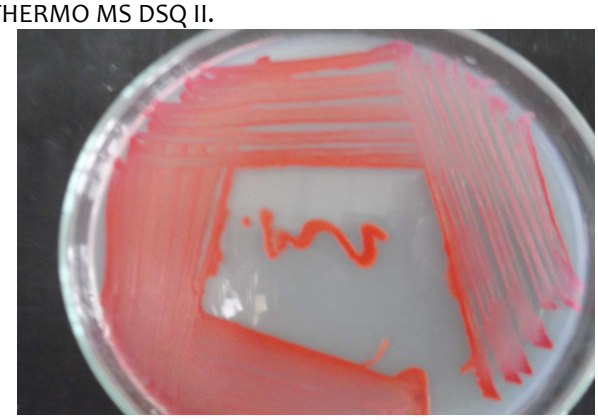

Fig.1: Growth of serratia in nutrient agar

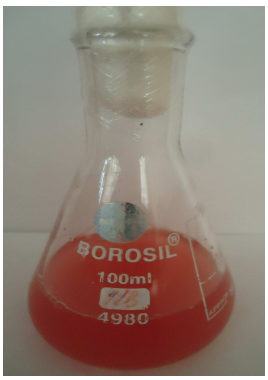

Fig.2: pigment production in nutrient broth

Fig.2: pellet formation after centrifugation

Fig.3: presumptive test for prodigiosin

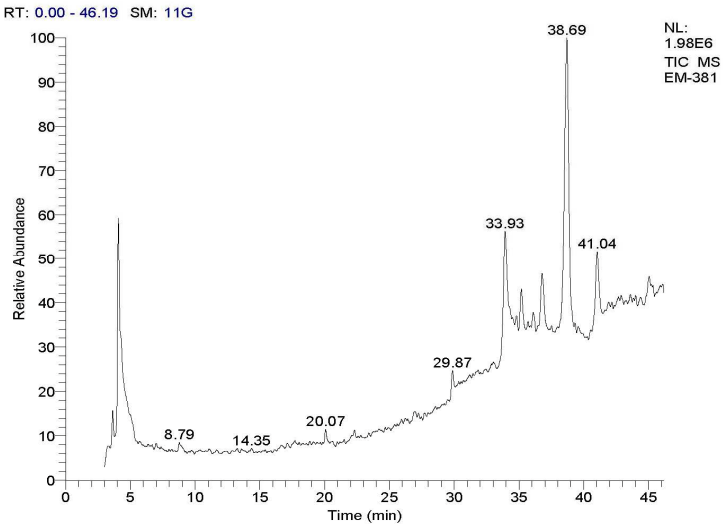

Fig.4: GC MS Graph

RESULTS AND DISCUSSION

The soil isolated from the garden at our campus gave a red coloured colonies which was identified as Serratia sps., by biochemical tests[11] and the organism is more confirmed as *S. marcescens* by the protein profiling carried out by MALDI-TOF mass spectrophotometry (fig.1). The pigment production is observed only after 24-48 hrs of incubation (fig.2) and the intensity of the color increased upon prolonged incubation which exactly matches with the results [12]. The presumptive test for prodigiosin shows the red and yellow colour in acidic and alkaline condition (fig.3) which coincides with the study of Giri *et al.*, 2004[13].The pigment production was enhanced when the nutrient broth was supplemented with 1% of glycerol in the medium. Which exactly matches with the results (Giri *et al.*, 2004)[13]. It has been discovered that prodigiosin possesses antibacterial, antifungal, antiprotozoal [14], cytotoxic [15] antitumor[16], antimalarial, antidiabetic, and anti-inflammatory properties. Surprisingly the crude methanolic extract when subjected to GC-MS analysis it revealed many other biologically important compounds in it. (Table.1). The crude methanol extracts which is analysed by GC-MS shows most of the compounds are alkaloids and are of high therapeutic importance. These alkaloids and quinoline compounds from other sources have been studied earlier for antimicrobial properties and potent anticancer property[17]. To the best of our knowledge and literature survey, this is the first study to report the production of such products from *S. marcescens*. The therapeutically important secondary metabolites reported to be produced by *S. marcescens* reflect a new area of interest in chemical and pharmaceutical research.

Abbreviations Used:

MALDI-TOF: Matrix-assisted laser desorption ionization time-of-flight mass Spectrometry
GC-MS: Gas chromatography–mass spectrometry
RPM: Revolutions per minute
He: Helium gas
OD: Optical density

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