Indian J. Applied & Pure Bio. Vol. 39(3), 1857-1869 (2024). **ISSN: 0970-2091**
A web of Science Journal

Development of Cotton fabric with extracted pigment from *Serratia marcescens* for Medical application

Sarah Thomas[1] and N. Vanitha[2]

[1,2]Department of Microbiology, Hindusthan College of Arts & Science, Coimbatore - 641028 (India)

Abstract

Serratia marcescens is a gram-negative, short rod-shaped, facultative anaerobic bacterium. it is commonly found in moist soil regions, grows at room temperature of about 25ºC to 30ºC, and is characterized morphologically and biochemically. Further identification was done by 16 srNA sequencing using BLASTN and confirmed to be *Serratia marcescens*. *S. marcescens* is noted for producing secondary metabolites called prodigiosin, a bioactive compound. the extraction process was carried out through the fermentation of prodigiosin pigment. The pigment was extracted by thin layer chromatography and the sample was purified by column chromatography. The purified sample was subjected to analysis of the functional group through FTIR analysis. The prodigiosin pigment was coated onto the cotton fabrics and used as an antimicrobial, anti-oxidant, and the physical strength of the coated fabrics like thickness, air permeability, and stain resistance test. Prodigiosin has a defined role in the physiology of the strains in which it is produced and is also naturally used for wounds and medicinal properties.

Key words : *Serratia marcescens,* prodigiosin, antimicrobial activity, 16 srNA sequencing.

Pigment provides promising technological tools through which a broad range of novel materials can be synthesized and seem to have huge potential in biomedical and life science. Bacterial pigments are colored secondary metabolites secreted during stress conditions. Bacterial pigments have diverse applications in various industries such as the pharmaceutical, food, and textile industries. Bacterial pigments are bacterial secondary metabolites that usually have bright colors and some special properties (e.g., antimicrobial, antioxidative, etc.). In addition to their high production yield, these special properties led scientists to research and develop methods for utilizing bacterial pigments in textile dyeing. This study presents the current state of this field of research, with a focus on the dyeing

[1]M.Sc. Microbiology, [2]M.Sc., M.Phil., Ph.D., Professor,

potential of bacterial pigments for different types of textile material.

Natural products either synthesized or secreted by organisms represent one of the critical sources of potential medicinal use. One of these less significant molecular weight natural products secreted by organisms and have no demonstrable function on the secreting cells are known as secondary metabolites that include pigments, steroids, enzymes, and antibiotics. These products are widely used for treatment. Bioactive pigments are obtained from plants, microorganisms, and many other sources.

Bioactive pigments produced by microorganisms are mostly preferred when compared with plants because of their stability and availability. In daily life, both natural pigments and synthetic dyes have been widely used in various fields such as foods, feeds, textiles, paper, printing inks, cosmetics, pharmaceuticals, etc.

Here we report simple and eco-friendly method textiles are one of the rapidly growing industries worldwide, that utilizes enormous amounts of synthetic dyes. The effluent from these textile industries poses a serious threat to the environment, which is often very difficult to treat and dispose of. Based on the study, an attempt was carried out to isolate the pigment-producing Serratia marcescens from the soil samples. The red pigment-producing bacteria was isolated and characterized using nutrient broth.

The highest yield of the pigment was obtained from the nutrient broth, which was found to be the best and cheapest for the prodigiosin pigment production from *Serratia marcescens*. The pigment was dyed on cotton cloth which exposed a good color tone. The pigment also has antimicrobial activity. In large-scale production, the pigment will make it an alternative to chemical dyes and further study can carried out in bedridden patients to treat bed sore and wound healing properties. For the preparation of the manuscript relevant literature[1-26] has been consulted.

Collection of soil sample :

The soil sample was collected from G.V Residency near Sowripalayam (latitude 11.019346 and longitude 77.01104) Coimbatore, Tamil Nadu. About 15g of samples were collected by scrapping to a depth of 1-3 cm using a sterile spatula. The samples were placed in a sterile plastic bag and stored at 4°C.

Isolation of *Serratia* sp *:*

The soil sample was serially diluted and 0.1 ml of diluted sample from each dilution was spread over the nutrient agar plates. Then the plates were incubated at 37°C, after 24 hrs. The distinct colonies were taken and identified by biochemical characterization.

Morphological identification :

It includes both macroscopic observation and microscopic observation of colonies on the agar plates. Macroscopic characterization of the colonies was performed based on the following parameters such as shape, size, and color based on the report Bergey's Manual of Determinative Bacteriology.

Microscopic examination :

To confirm the identification of sample was performed by using gram staining

Biochemical test :

The different biochemical test was carried out such as methyl red test, Voges Proskauer test, citrate, triple sugar iron agar test, urease test, catalase test, and oxidase test.

Fermentation :

The nutrient broth was prepared in 250 ml inoculated with the isolated strain and incubated at 30°C for 7 days.

Pigment extraction :

The nutrient broth inoculated with the isolated organism was observed for pigment production. The fermented broth was centrifuged at 5000 rpm for 10 mts. Debris was removed and the supernatant was taken in centrifuge tubes.

Thin layer chromatography :

Thin-layer chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminum foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material is usually used silica gel. On completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (R)expressed as:
Rf = distance traveled by the solute/ distance traveled by the solvent.

Column chromatography :

Take 25g of silica gel G250 and 25 ml of distilled water and make a slurry. A cotton wool is placed at the bottom of the column. After packing, the preparation was poured into the column and kept for saturation for 2 hrs. After saturation, the sample was loaded in the packed column slowly. 3 sequential fractions were collected at intervals of 30 minutes.

Physio-chemical analysis of prodigiosin :

UV-visible spectroscopy :

UV spectroscopy is a technique used to study how molecules absorb ultraviolet and visible light. Extracted pigments were measured and the absorption spectra were scanned at the wavelength of 400 – 700nm.

FTIR analysis :

FTIR stands for Fourier transform infrared spectroscopy" and it is the most common form of infrared spectroscopy. All infrared spectroscopies act on the principle that when infrared radiation (IR) passes through the sample, some of the radiation is absorbed. The radiation that passes through the sample was recorded. By employing the FTIR spectro-photometer, structural information can be elucidated from its various vibrational modes. The extracted pigment was directly used for FTIR analysis.

Coating over the fabrics :

The 2 types of fabrics were cut into 1×7 cm long and 3 times coated with purified pigment and dry.

Antioxidant activity :

Antioxidant activity was carried out by 1, 1- diphenyl- 2- picrylhydrazyl (DPPH) assay using a modified method of Brand-Williams. DPPH (oxidized form) is a stable free radical with a purple color. In the presence of an antioxidant that can donate an electron to DPPH radical decays, and the change in absorbance at 520 nm was followed spectro-

photometrically. Antioxidant activity was done with extracted prodigiosin pigment for the calculation of radical scavenging % and IC_{50} activity by DPPH assay.

Procedure :

Preparation of DPPH-2,2-diphenyl-1picrylhydrazyl 1M DPPH – 4mg DPPH and 100 ml of 99% methanol and keep it in cool conditions (Tris HCl buffer (pH-7.4)). The total free radical scavenging capacity of the prodigiosin extract sample was estimated using the stable DPPH radical, which has an absorption maximum of 430 nm. A solution of the radical is prepared by dissolving 4 mg DPPH in 100 ml methanol. The aqueous extract of prodigiosin of 100µl, 200µl, 300µl, 400µl, and 500µl was added to 3 ml methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 30 min in a dark place. The absorption in the reaction mixture was measured calorimetrically. The absorbance of the DPPH radical without antioxidants, *i.e.*, blank was also measured. All the determinations were performed in triplicate.

• Radical scavenging activity/inhibition (%) = (Ab-As)/Ab x 100

Antibacterial activity :

The evaluation of the antibacterial activity, for pigment, was made based on the halo formed around the edges of the samples and the bacteria growth under the samples. For this method, fabric samples of 1×7 cm were used. Once the fabrics were cut, it was dipped in the extracted pigment and placed on the plate against the test organism. The plates were incubated at 27°C for 24 hours.

Stain resistance test :

The test was carried out with citric acid and with the coated bleached and unbleached cotton fabrics. The citric acid concentration was taken as 1%, 2%,3%,4% and 5%. The coated fabric was cut into 1*1 (length * breadth) and placed in the center of the Petri plate. Immersed in citric acid with distilled water for the observation of dye. The observed days were recorded.

Thickness test :

It determines the thickness standard and thickness of the coated fabric (ASTM-D- 1777)

Air permeability test :

Measurement of air permeability (ASTM – D- 737-2004)

Procedure:

The air was passed through the finished fabric and control fabric and the average rate of flow in cubic cm/sec. Through the test area, 5.07cm^2 was computerized. The airflow (The volume of air in cubic cm passed per second through 1cm^2 of the fabric at a pressure difference of 1cm of water) was calculated by dividing the mean flow by 5.07

16s rRNA Gene Sequencing :

Genomic DNA isolation:

DNA isolation from Microbial samples was done using the Biobee Spin EXpure Microbial DNA isolation kit developed by Bogar Bio Bee stores Pvt Ltd.,

PCR Protocol :

Polymerase Chain Reaction (PCR) is a process that uses primers to amplify specific cloned or genomic DNA sequences with the help of a very unique enzyme. PCR uses the enzyme DNA polymerase that directs the synthesis of DNA from deoxynucleotide substrates on a single-stranded DNA template. DNA polymerase adds nucleotides to the 3‘ end of a custom-designed oligonucleotide when it is annealed to a longer template DNA. Thus, if a synthetic oligonucleotide is annealed to a single-stranded template that contains a region complementary to the oligonucleotide, DNA polymerase can use the oligonucleotide as a primer and elongate its 3‘ end to generate an extended region of double-stranded DNA.

Bioinformatics protocol :

1. The 16s rRNA sequence was BLAST using the NCBI blast similarity search tool. The phylogeny analysis of the query sequence with the closely related sequence of blast results was performed followed by multiple sequence alignment.
2. The program MUSCLE 3.7 was used for multiple alignments of sequences. The resulting aligned sequences were cured using the program Gblocks 0.91b.These Gblocks eliminate poorly aligned positions and divergent regions (removes alignment noise). Finally, the program PhyML 3.0 aLRT was used for phylogeny analysis and HKY85 as a Substitution model.
3. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data while being one order of magnitude faster. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data while being one order of magnitude faster. The program Tree Dyn 198.3 was used for tree rendering.

Isolation of *Serratia* sp. *:*

The collected soil samples were subjected to *Serratia* sp culture isolation. 1g of soil sample added with 10 ml of distilled water. *Serratia* sp colonies were isolated by serial dilution method (till 10^6) using a nutrient agar medium. Then the plates were incubated at 37^{o}C. After 24 hrs., The distinct colonies were taken and identified by biochemical characterization. (Figure 1).

Morphological identification of Serratia sp:

The colonies appeared smooth, circular and raised and typically pigmented a red nature. (Figure 2) Similar to the study of which also showed the similar appearance and nature of the *Serratia* sp.

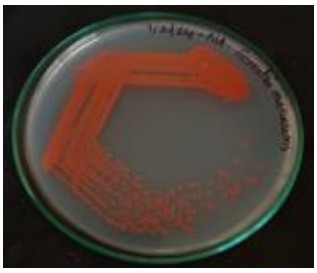


Fig. 1 Streak plate of *Serratia* sp

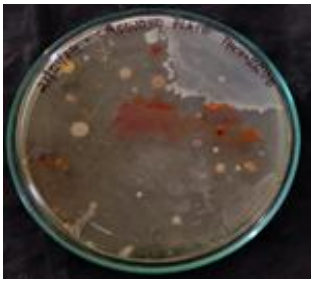


Fig. 2 Crowded plate of *Serratia* sp

Microscopic examination :

Microscopic examination of gram-stained *Serratia marcescens* isolates was performed. The *Serratia marcescens* exhibited purple rod-shaped bacilli under 100x magnification which depicts the gram-negative bacteria. Similar studies were done to confirm the nature of *Serratia marcescens* earlier by isolated from water and wet soil and confirmed them as *Serratia marcescens* by their nature and stained surfaces. (Figure 3)

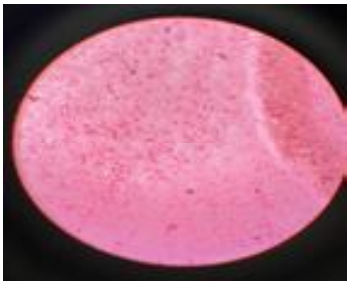


Figure 3. Gram staining of *Serratia* sp

Biochemical Characterization :

The biochemical tests were performed for the identification of *Serratia* marcescens including major tests are carried out. (Table-1)

Table-1. The biochemical test for *Serratia* marcescens

1	Methyl red test	Negative
2	Voges Proskauer test	Positive
3	Citrate test	Positive
4	Urease test	Positive
5	Triplesugar iron agar test	Acid butt, alkaline alkaline slant with gas production
6	Oxidase test	Negative
7	Catalase test	Positive
8	Gelatin hydrolysis test	Positive

The various biochemical tests were performed and it was found that the isolated strain belonged to genus *S. marcescens* by figures showing methyl red negative, Voges Proskauer positive, citrate positive, triple sugar iron reaction showed yellow color butt (acid) and red color slant (alkaline) with gas production, urease with slow positive, gelatin test shows liquefaction after refrigeration it utilizes the enzyme gelatinase and shows positive, oxidase test negative, and catalase test shows positive with immediate air bubble formation were performed according to the procedures of Aneja[2] and the genus of organism was characterized by Bergey's Manual of Determinative bacteriology.

Fermentation :

The production of pigments from the selected organism was carried out using Nutrient broth and sterilized at 121°C for 15 minutes at 15 lbs pressure. The heavy inoculum was inoculated in 250ml of the broth and incubated for 48 hours at 28°C to determine the pigment production. (Figure 5) Similar studies were done to confirm with and identified as same pigment optimization in nutrient medium.

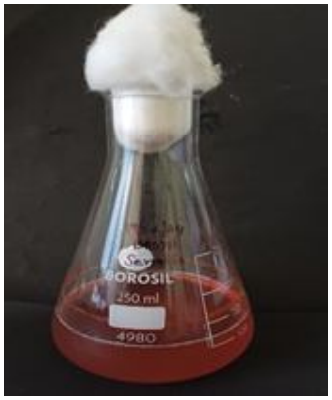


Figure 4. Fermentation on nutrient broth

Extraction of prodigiosin pigment :

The fermented broth with distilled water was centrifuged at 5000 rpm for 10 mins. Debris was removed and the supernatant was taken in centrifuge tubes.

Thin layer chromatography :

The Rf value of *Serratia marcescens* is 0.8 indicates the presence of prodigiosin.

Column chromatography :

After packing, the preparation was poured into the column and kept for saturation for 2 hrs. After saturation, the sample was loaded in the packed column slowly. 3 sequential fractions were collected at intervals of 30 minutes

Figure 5. Fractions of Purified pigments

Physio-chemical analysis :

UV visible spectroscopy :

The UV Spectroscopy was characterized for the prodigiosin pigment. The peaks were observed in the wavelength range of 400 – 700 nm. The peak absorbance for the prodigiosin pigment at 0.4607 in wavelength of 696 nm. (Table-2).

Table-2. Absorbance in UV Spectroscopy

Wavelength (nm)	Absorbance
696	0.4607

Fourier Transform infrared spectroscopy :

The FTIR spectra of the pigment are in the range of 4000 to 400 cm1. For sample presence of multiple peaks 3740.26, 3653.48, 2359.48, 1631.48, 1530.24 was confirmed. The broadened appearance of intense bands with the O-H bond group was confirmed at 3740.26 and 3653.48 cm^{-1}. The peak bands at 1631, 1530, and 1484 cm^{-1} represent the C=O stretch of COO and CHO moiety, and the C-O stretching peak was confirmed at 1631.48 cm^{-1}. Thus the FTIR results confirm the prodigiosin pigment.

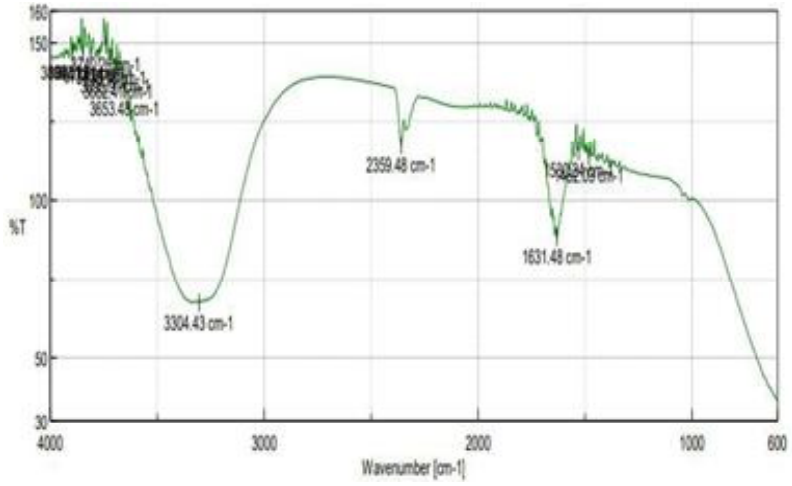


Figure 6. Graph of FTIR

Table-3. FTIR of Prodigiosin pigment

Peak	Sample pigment	Standard	Appearance	Functional group	Compound
Peak 1	3653	3617	Medium	OH, NH	Alcohol, Amine
Peak 2	3682	3417	Medium	OH, NH	Alcohol, Amine
Peak 3	3304	3417	Medium	OH, NH	Alcohol, Amine
Peak 4	1631	1619	Strong	C=O, C=C	Conjugated ketone, Alkene

Antioxidant activity :

DPPH Assay :

Table-4. Antioxidant activity of prodigiosin pigment

Concen-tration	Control absorbance at 517nm	Sample absorbanceat 517nm	%RSA	IC_{50}
100	1.025	0.91	11.21	73.3969
200	1.025	0.84	18.04	173.3969
300	1.025	0.75	26.82	273.3969
400	1.025	0.64	37.56	373.3969
500	1.025	0.58	43.41	473.3969

The antioxidant activity of Prodigiosin pigment is measured. Different concentration is taken and measured. (Figure 12) 100, 200, 300, 400, and 500 were taken, and % RSA 11.21, 18.04, 26.82, 37.56, and 43.41 respectively.

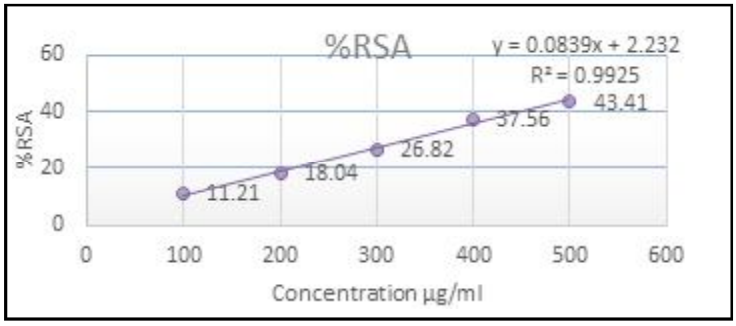


Figure 7. The graphical representation of DPPH Assay

Coating on cotton fabrics (Dip dry method) :

The two different kinds of coated 40s cotton fabrics bleached and unbleached were cut into pieces (1*7) and the pieces were tested for thickness, and air permeability at SITRA, Coimbatore, and results are observed below

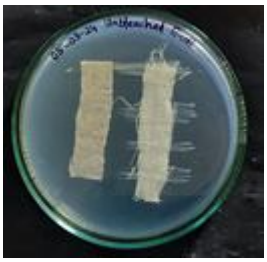
Figure 8a. Unbleached coated fabric

Figure 8b. Bleached coated fabric

Antibacterial activity :

Swatches test :

The antibacterial activity test was carried out for the development of coated pigment and uncoated pigment (control) in fabrics against *Staphylococcus aureus* and *Escherichia coli*. A zone of clearance was observed.



Unbleached *E. coli*

Unbleached *S. aureus*

Bleached *S. aureus*

Bleached *E. coli*

Table-5. Antibacterial activity of coated fabric

Organisms	Bleached fabric	Unbleached fabric
Escherichia coli	1mm	1mm
Staphylococcus aureus	3mm	3mm

The zone of inhibition was observed. The developed coated fabrics are more sensitive to *Staphylococcus aureus than Escherichia coli.*

Stain resistance test :

Stain resistance (AATCC,175-2005 & AATCC TM 130) is done to evaluate the effectiveness of fabrics using citric acid to retain the stains. The ability of coated fabrics to withstand permanent discoloration due to the action of stains, the property depends partly upon the chemical nature of the fabric and is improved by pro-prientary treatments. The assessment of stain resistance is shown in (Table 6) it shows mild changes occur on day 4 and day 5 of 0.4% and 0.5% concentration. The coated fabrics retain stains, indicating high resistance in the prodigiosin pigment.

Table-6. Citric acid test for coated fabrics

Concen-tration of citric acid	Day 1	Day 2	Day 3	Day 4	Day 5
0.1%	No change	No change	No change	No change	No change
0.2%	No change	No change	No change	No change	No change
0.3%	No change	No change	No change	No change	No change
0.4%	No change	No change	No change	Mild change	Mild change
0.5%	No change	No change	No change	Mild change	Mild change

Thickness test :

The thickness of coated fabrics was measured according to the standard method (ASTM- D - 177), the coated fabric had a 0.23mm increased thickness when compared to the control fabric 0.2mm, and the interference was good at the pigment-coated fabric. (Table-7).

Table-7. Citric acid test for coated fabrics

Sample	Thickness standard	Thickness of coated fabric	Interference
Unbleached fabric	**0.2mm**	**0.23mm**	**Good**

Air permeability test :

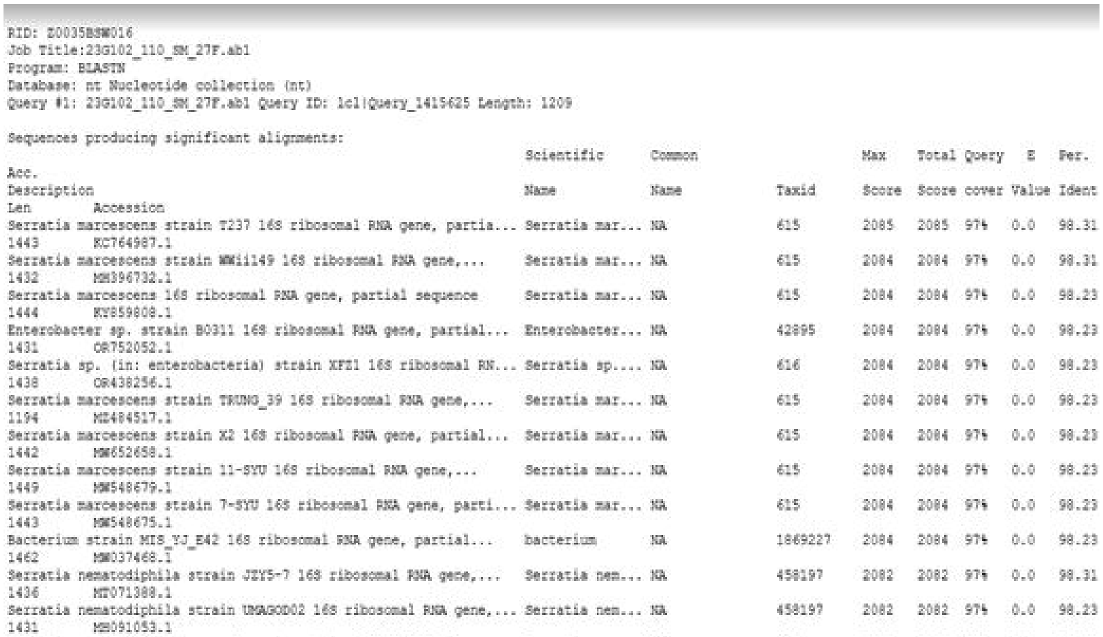
The air permeability of the Pigment-coated fabric (89.3 cc/cm.sq/sec) was noticed to be much lower than the uncoated fabric (92.8 cc/cm.sq/sec). The fabric thickness increases in the Pigment-coated cloth, so the air permeability decreases. The finished fabric had lesser air permeability when cpared with non-coated fabric.

Table-8. Air permeability test for coated fabric

Sample	Properties	Control fabric	Pigment coated fabric
Unbleached fabric	**Air permeability (cc/ cm.sq/sec)**	**92.8cm**	**89.3cm**

16s rRNA Sequencing :

Comparison of 16s rRNA sequence of strain T237 with closely related taxa retrieved that the strain belonging to the genus *Serratia* (Phylum Proteobacteria). In phylogenetic analysis, the highest sequence similarity (97%) was observed in T237. The finding revealed the presence of strain T237 as *Serratia marcescens*.



RID: Z0035BSW016
Job Title:23G102_110_SM_27F.ab1
Program: BLASTN
Database: nt Nucleotide collection (nt)
Query #1: 23G102_110_SM_27F.ab1 Query ID: lcl|Query_1415625 Length: 1209

Sequences producing significant alignments:

Description	Scientific Name	Common Name	Taxid	Max Score	Total Score	Query cover	E Value	Per. Ident	Acc. Len	Accession
Serratia marcescens strain T237 16S ribosomal RNA gene, partia...	Serratia mar...	NA	615	2085	2085	97%	0.0	98.31	1443	KC764987.1
Serratia marcescens strain WWi149 16S ribosomal RNA gene,...	Serratia mar...	NA	615	2084	2084	97%	0.0	98.31	1432	MH396732.1
Serratia marcescens 16S ribosomal RNA gene, partial sequence	Serratia mar...	NA	615	2084	2084	97%	0.0	98.23	1444	KY859808.1
Enterobacter sp. strain B0311 16S ribosomal RNA gene, partial...	Enterobacter...	NA	42895	2084	2084	97%	0.0	98.23	1431	OR752052.1
Serratia sp. (in: enterobacteria) strain XFZ1 16S ribosomal RN...	Serratia sp....	NA	616	2084	2084	97%	0.0	98.23	1438	OR438256.1
Serratia marcescens strain TRUNG_39 16S ribosomal RNA gene,...	Serratia mar...	NA	615	2084	2084	97%	0.0	98.23	1194	MZ484517.1
Serratia marcescens strain X2 16S ribosomal RNA gene, partial...	Serratia mar...	NA	615	2084	2084	97%	0.0	98.23	1442	MW652658.1
Serratia marcescens strain 11-SYU 16S ribosomal RNA gene,...	Serratia mar...	NA	615	2084	2084	97%	0.0	98.23	1449	MW548679.1
Serratia marcescens strain 7-SYU 16S ribosomal RNA gene, parti...	Serratia mar...	NA	615	2084	2084	97%	0.0	98.23	1443	MW548675.1
Bacterium strain MIS_YJ_E42 16S ribosomal RNA gene, partial...	bacterium	NA	1869227	2084	2084	97%	0.0	98.23	1462	MW037468.1
Serratia nematodiphila strain JZY5-7 16S ribosomal RNA gene,...	Serratia nem...	NA	458197	2082	2082	97%	0.0	98.31	1436	MT071388.1
Serratia nematodiphila strain UMAGOD02 16S ribosomal RNA gene,...	Serratia nem...	NA	458197	2082	2082	97%	0.0	98.23	1431	MH091053.1

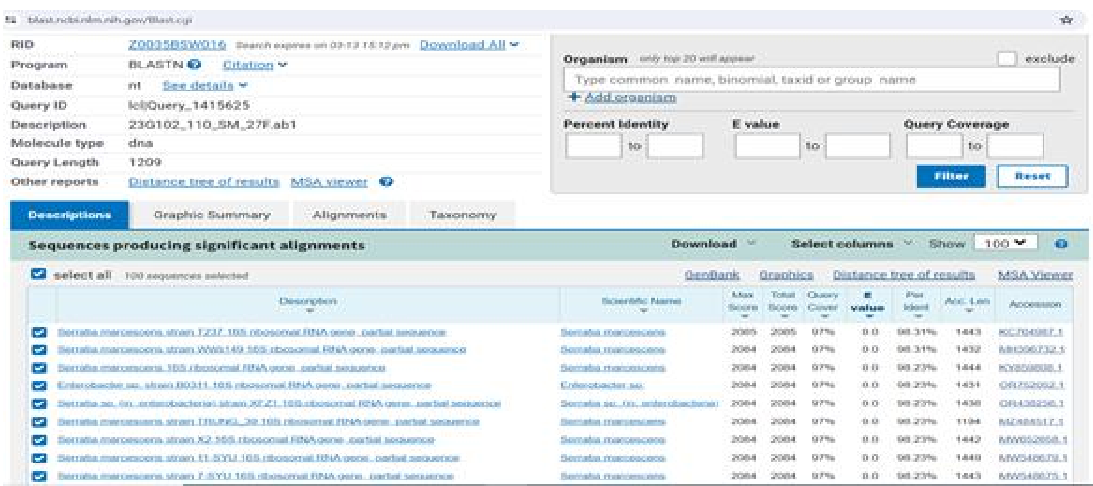


Figure 10a and 10b - 16 s RNA sequencing of *Serratia marcescens* by using BLASTN

Summary and conclusion

Prodigiosin is a naturally occurring red pigment by *Serratia marcescens* and it has biomedical applications. The pigment was produced in a nutrient broth medium for much pigment production. The maximum pigment was produced at 28! and 30! in nutrient broth. The pigment separation and its Rf value were determined by the thin layer chromatography

method using silica gel plates. Prodigiosin represented the value of 0.8 spotted and that of pink color fractions were collected from column chromatography during three sequential fractions. the absorbance value of prodigiosin was determined by running on UV-visible spectroscopy with absorbance at 696nm. FTIR was analyzed to confirm the functional groups and stretching peaks that appeared strong peak at 1631.48 cm^{-1} and weak at 3304.43 cm^{-1}. Thus the FTIR results confirm the prodigiosin pigment with standard. The antioxidant activity was performed using DPPH assay and pigment shows antioxidant properties for free radical scavenging. Prodigiosin a secondary metabolite produced by *Serratia marcescens* was found to be anti-bacterial. Different zone of clearance was observed on test organisms from *Staphylococcus aureus* and *Escherichia coli,* the coated bleached cotton fabric was comparatively larger than unbleached fabrics. Coated fabrics were performed for thickness and air permeability to show the properties of prodigiosin pigment. 2 Comparison of 16s rRNA sequence of strain T237 with closely related taxa retrieved that the strain belonging to the genus *Serratia* (Phylum Proteobacteria). In phylogenetic analysis, the highest sequence similarity (97%) was observed in T237.

The present study investigated coated fabrics with two different kinds of bleached and unbleached fabrics. The study also describes the antioxidant properties and anti-bacterial. In the future, it can act as a safe biological prodigiosin pigment known to possess wide medicinal applications for bedridden patients for acting as bed sore and wound healing. Hence, further study will focus on the medical applications of prodigiosin.

References :

1. Ahmad WA, WYW Ahmad, ZA Zakaria, and NZ Yusof (2012) Application of bacterial pigments as colorant. In: Application of bacterial pigments as colorant. Springer, pp 57–74.
2. Aneja, K.R., Experiments in Microbiology, Plant Pathology and Biotechnology, New Age International Publishers, New Delhi, 2003; 245-249.
3. Azeko, S.T., O.S. Odusanya, K. Malatesta, N. Anuku, and W. O. Soboyejo, (2016). *Advanced materials research, 1132:* 238-251.
4. C.H. Kim, S.W. Kim and S.I. Hong (1999). *Process Biochemistry 35*(5): 485-490.
5. Carbonell T, T Della Colleta, A Yano, C Darini, C E Levy, and B. Fonseca (2000). *A survey of the last 2500 years. Chem Int Ed Engl 42:* 3582-3603.
6. Chauhan, R., A. Choudhuri and J. Abraham (2017). *Asian journal of pharmaceutical and clinical research,* 279-283.
7. Devi, M., E. Ramakrishnan, S. Deka, and D. P. Parasar, (2024). *Journal of Microbiological Methods,* 106907.
8. El-Bialy, H. A., and S. A. Abou El-Nour, (2015). *Annals of microbiology, 65*(1): 59-68.
9. Giri, A.V., N. Anandkumar, G Muthukumaran and G. Pennathur (2004). *BMC. Microbiol, 4:* 11-18.
10. Gulani, C., S. Bhattacharya, and A. Das, (2012). *Malays J Microbiol, 8*(2): 116-122.
11. Harned, R.L. (1954). *Applied Microbiology, 2*(6): 365-368.
12. Ibrahim, D., T. F. Nazari, J. Kassim, and S. H. Lim, (2014). *Journal of Applied Pharmaceutical Science, 4*(10): 001-006.

13. Ibrahim, G. S., S. A. Abdelhamid, E. A. Elmansy, M. M. Asker, and N. Fatma, (2023). *Biocatalysis and Agricultural Biotechnology, 54:* 102932.
14. Joshi V, D Attri, A Bala and S. Bhushan (2003). *J Biotechnol. 2*(362)
15. Kamble, K.D., and V.D. Hiwarale (2012). *International journal of environmental sciences, 3*(1): 631-638.
16. Kim CH, SH Kim, and SI. Hong (1998). *Korean J Appl Microbiol Biotechnol.*
17. Lapenda, J.C. L., V. P. Alves, M. L. Adam, M. D. Rodrigues, and S. C. Nascimento, (2020). *Indian journal of microbiology, 60:* 182-195.
18. Liu, X., Y. Wang, S. Sun, C. Zhu, W. Xu, Y. Park and H. Zhou, (2013). *Preparative Biochemistry and Biotechnology, 43*(3): 271-284.
19. Mehta, M. (2018). Screening and isolation of naturally occurring microbial pigments their characterization and applications.
20. Mekhael, R. A. M. I. N.A. and S.Y. Yousif (2009). *J Duhok Univ, 12*(1): 268-274.
21. Nakashima, T., M. Kurachi, Y. Kato, K. Yamaguchi and T. Oda, (2005). *Microbiol Immunol. 49:* 407–415.
22. Neely, A. N. (2000). *The Journal of burn care & rehabilitation, 21*(6): 523-527.
23. Nerurkar, M., J. Vaidyanathan, R. Adivarekar and Z. B. Langdana, (2013). Use of a natural dye from *Serratia marcescens* subspecies Marcescens in dyeing of textile fabrics. Octa *Journal of Environmental Research, 1*(2).
24. Pallath, N., S. Johnson, E. Paul, and R. TA, (2023). *Environmental Quality Management*.
25. Paul, T., T.K. Bandyopadhyay, A. Mondal, O. N. Tiwari, M. Muthuraj, and B. Bhunia, (2020). *Biomass Conversion and Biorefinery,* 1-23.
26. Pradeep, B., P. Stanley, *et al.,* (2013) *Asian Journal of Pharmaceutical and Clinical Research. 6*(1): ISSN - 0974-2441.