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Isolation, Optimization and Application of Pigment Producing Bacteria from Soil

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Abstract

Color is the most crucial aspect of any item since it improves its attractiveness and acceptability. Different dyes are available in the market which makes their way to different industries. Synthetic colors are more reliable and less expensive than natural colors, but due to health hazards, few remain prohibited. In dye, food, pharmaceutical, cosmetic industries, widely use innocuous pigments from microbes. The work focuses on isolating the pigment-producing bacteria from soil and investigate several parameters that influence pigment synthesis. Bio colorant derived from A1 and A2 were applied on cotton fabric with pigment extracted by the solvent method. Different temperature, pH, incubation, and static and shaking conditions were investigated in this study. Biochemical and Molecular analysis identified both isolates A1 and A2 as *Pseudomonas aeruginosa* and *Salinococcus roseus.* This work demonstrated pigment production was good under neutral pH and at shaking conditions. Both isolates showed different incubation times for pigment synthesis. Only *Pseudomonas aeruginosa* exhibited antibacterial action against the test pathogens *E.coli and Staphylococcus aureus.*

Keywords: Biocolorant, pigment production, eco-dyeing, antimicrobial activity, Characterization.

1. Introduction

Colored chemicals that come in different hues are known as Pigments. Hues are used for aesthetic reasons and to attract consumers. In the food, apparel, and cosmetics industries, pigments are commonly used as coloring agents. Synthetic coloring agents have been broadly applied since the 1850's due to their simplicity of manufacture, lower cost, improved coloring capabilities, and a

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minimal amount of coloring required [1,2]. Synthetic dyes' have hyper-allergenicity or carcinogenicity consequences, hence prompted more investigation into natural colors. Ores, insects, plants, and bacteria are all sources of natural pigments. As they are available throughout the year, bio pigments are preferred over plants [8]. Bacterial colors are an excellent natural resource alternative to manufactured pigments due to their distinct characteristics [3]. Bacterial pigments are also one of the newest topics of study, with exciting possibilities for a variety of applications. Bio colorant is a coloring agent generated from living organisms like Anthocyanidin, carotenoids, prodigiosin, and other pigments. To prevent the perils of artificial dyes, a growing interest is seen in creating procedures for making pigments from natural sources all around the world [4].

The pigment is a substance made up of minute particles that are insoluble in the medium and commonly applied as a colorant, additives, antioxidants, color intensifiers, and other things in the food industry. Water-soluble pigments are available in a variety of colors [5].

Marketing and synthesis of carotenoid pigment from vegetables are influenced by seasonal and geographic variation [6]. The nontoxic pigments produced by microorganisms make them ideal for dyes, foods, pharmaceuticals, and other industrial applications [8]. Furthermore, natural colorants derived from microorganisms have therapeutic value as antioxidants, antimicrobials, additives, color intensifiers, and anticancer agents, in addition to being cost-effective. *Staphylococcus aureus,* shows Golden Yellow, *Agrobacterium aurantiacum,* with Pink-red *Chromobacterium* with Purple red, *Serratia marcescens,* with Creamy, *Bacillus spp.* with yellow, colored pigments were observed [7].

The red basidiomycetous yeast and the green algae *Xanthophyllomyces dendrorhous* and *Heamatococcus pluvialis* are the sources of natural astaxanthin [9]. *Penicillium oxalicum*, a fungus strain that can create a red colorant, can be used in the food and cosmetic sectors [10]. Environment parameters will impact microbial growth and survival, such as pH, salt content, ambient temperature, nutrient factors. In general, stressful circumstances influence microbial growth and boost their ability to create secondary metabolites such as enzymes, pigments, and antibiotics. Furthermore, some bacteria may experience a mutation that alters their phenotypic and allows them to survive and reproduce. Microbes should have mechanisms to maintain low mutation arising from the detrimental effects of mutation produced by stress factors [11]. The present work focuses on identifying pigment-producing bacteria from soil, optimization, pigment synthesis, extraction of the pigments, and their application.

2. Materials and Methodology

2.1 Collection of sample

Soil samples were taken from Edakkara, Malappuram district under various soil conditions, like riverbed side, garden, roadside, and workshop soil. The collected samples were placed in a sterile polythene bag and labeled appropriately.

2.2 Isolation of Pigment-Producing Bacteria Collected soil samples were used for serial dilution up to $10^{-9.}$, 10^{-4} to 10^{-9} were plated on nutrient Agar plates were incubated at 37°C for 24 hours. After which, pigmented colonies alone were propagated for 48 hours at 37^0 C. Pure culture of well isolated colonies were obtained [12]

2.3 Identification

The colonies were further subjected to preliminary identification based on morphology and biochemical tests [13].

a. Molecular Characterization of Selected Isolates Using 16S rRNA Sequence Analysis. Molecular identification of selected bacteria was done using 16S rRNA gene sequence.

i) Genomic DNA isolation-

Genomic DNA was isolated according NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions.

A part of cultures are taken in a microcentrifuge tube. 180 µl of T1 buffer and 25 µl of proteinase K was added and incubated at 56°C in a water bath until it was completely lysed. After lysis, 5 µl of RNase A (100 mg/ml) was added and incubated at room temperature for 5 minutes. 200 µl of B3 buffer was added and incubated at 70°C for 10 minutes. 210 µl of 100% ethanol was added and mixed thoroughly by vortexing. The mixture was pipetted into NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11000 x g for 1 minute. The NucleoSpin® Tissue column was transferred to a new 2 ml tube and washed with 500 µl of BW buffer. Wash step was repeated using 600 µl of B5 buffer. After washing the NucleoSpin® Tissue column was placed in a clean 1.5 ml tube and DNA was eluted out using 50 µl of BE buffer. [14]

b. Agarose Gel Electrophoresis for DNA quality and Quantity check

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator and the image was captured under UV light using Gel documentation system (Bio-Rad). [30]

C) Amplification of 16SrRNA

PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X PCR buffer (100mM Tris HCl, pH-8.3; 500mM KCl), 0.2mM each dNTPs (dATP, dGTP, dCTP and dTTP), 2.5mM $MgCl_2$,1unit of AmpliTaq Gold DNA polymerase enzyme,0.1mg/ml BSA, 4% DMSO, 5pM of forward and reverse primers and template DNA [31].

Primers used in A1

Target	Primer	Direction	Sequence(5'➔3')
16S rRNA	16SRS-F	Forward	GACAACGCCCTCAGCATCACCAGC
	16SRS-R	Reverse	CGCTGGCCCATTCGCTCCAGCGCT

Primers used in A2

Target	Primer	Direction	Sequence(5'➔3')
16S RNA	16SRS-F	Forward	TTCCGGTTGATCCTGCC
	16SRS-R	Reverse	AAGGAGGTGATCCAGCC

The PCR amplifications were carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) followed by Agarose gel electrophoresis and unwanted primers were removed by Exo SAP-IT treatment.

2.4 Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1.

i. Identification and Phylogeny.

The final sequence was used for the analysis. The sequence similarity and phylogeny was done by BLAST of NCBI.

2.5 Optimization for pigment production.

i. Temperature:
Nutrient Broth was prepared in three sets. The nutrient broth were inoculated with bacterial isolates and each set was incubated at different temperature. The test tube set were incubated 27°C (room temperature) at 37°C (incubator), 47°C (water bath) and 57°C (Hot air oven), respectively, for 24 hours, to study the effect of temperature on the pigment production. Then OD was measured calorimetrically [32] [33].

ii. Effect of pH: Effect of pH on the pigment production by bacterial isolates, test tubes containing Nutrient broth were prepared in three sets. Prior to autoclaving the pH for each set of test tubes were adjusted to 6,7,8 and 9 respectively. The nutrient, from each set were inoculated with bacterial isolates and were incubated at 37ºC for 24hours to study the effect of pH on the pigment production. Then OD was measured [32] [33].

iii. Effect of incubation time: Nutrient broths were prepared in three sets. The nutrient broth were then inoculated with bacterial isolates and each set was incubated at different time i.e 24, 48, 72, 96 hours respectively, at 37ºC to study the effect of incubation time on the pigment production. Then OD was measured.

iv. Effect of shaking /static conditions: The bacterial isolates cultured were inoculated into sterile nutrient broth in conical flask and incubated at optimum pH, temperature, incubation time and under static as well as shaking conditions[15].

2.6 Production of Pigment

Pigment production was carried out based on method by isolates cultivated in Nutrient broth (NB). This experiment was done to determine the effect of bacterial growth and pigment production. A loopful colony of A1 and A2 was cultured in each Erlenmeyer flasks contains Nutrient broth (NB) incubated at 28°C for 24 hours as seed cultures. About 1 ml of each seed cultures was then transferred into each new Erlenmeyer flasks containing 250 ml of Nutrient broth (NB). All bacterial inoculum agitated vigorously at 120 rpm using incubator shaker at 28°C for 7 days.

Extraction of Pigment

Pigment produced by isolates were extracted based on method by centrifugation with condition set as 5000 rpm for 15 minutes by using centrifuge. The supernatant and pellet collected and extracted by using solvent.

Pigment Extraction from Pellet and supernatant

Pigment extraction from bacterial pellet was done using solvent extraction method. The pellet collected was re suspended with same amount of 100 ml acidified ethanol (4% of HCL and 96 ml of ethanol). The mixture of pellet and the solvent were vortexed and subjected to further centrifugation with condition set as 6000 rpm at for 15 minutes [34]

Pigment Extraction from Supernatant

Pigment extraction from supernatant was carried by solvent extraction method. The supernatant collected was resuspended with 100 ml acidified ethanol. The mixture of supernatant and the solvent were vortexed and subjected to further centrifugation with condition set as 6000 rpm for 15 minutes [34].

2.7 Antibacterial Activity

The pigments' antimicrobial activity was determined by agar well diffusion method against two human pathogens (*Escherichia coli* and *Staphylococcus aureus*). The spread plate technique was used to plate the sample on sterile Nutrient Agar plates, and wells were bored. The pigment was then added to the wells in the proper amount (30 ul) and incubated at 37° C for 24 hours, by measuring the zone of inhibition was determined [35].

2. Application of the Pigment

The application of extracted pigment was evaluated on cotton cloth. Cotton cloths cut in to pieces were soaked in 10 ml ethanol extract pigment of A1 and A2 taken in different petri plates and incubated at room temperature. White cloth material were taken as a control.

3. Results and Discussion

Because of the hazardous consequences of some synthetic dyes, the demand for natural colors is growing every day. Microbial pigments are an easy-to-find alternative to naturally generated colors. They have many advantages over other natural pigments, including growth, ease of processing, and weather resistance. Various biological activities like antioxidant, antibacterial, and anticancer capabilities were seen in a few pigments [16].

Four isolates were initially screened. Isolate showing high pigment (A1 and A2) were taken for further study. A1 showed, blue green pigment and A2 showed orange pigment. (Table 3.1)

Table 3.1-Pigment producing isolates

Sl. no	Isolate	Place of Soil	Pigment produced
1.	A1	Workshop	Blue green
2.	A2	Garden	Orange
3.	A3	River site	Yellow
4.	A4	Roadside	Yellowish orange

Table 3.1a Biochemical test results

Sl. no	Biochemical test	A1	A2
1.	Indole	_	_
2.	Methyl red test	_	_
3.	Citrate Utilization test	+	+
4.	Oxidase test	+	_
5.	Catalase test	+	+

The A1 was identified as *Pseudomonas aeruginosa* and A2 isolate was identified as *Salinococcus roseus* by biochemical (Table 3.1a) and 16S rRNA gene sequencing.

Table 3.2 shows OD at different temperature of two isolates

Pigment	Temperature	OD at 550nm
A1	control27	0.0
	37	0.10
	47	0.47
	57	0.28
		0.08
A2	control27	0.0
	37	0.03
	47	0.35
	57	0.29
		0.07

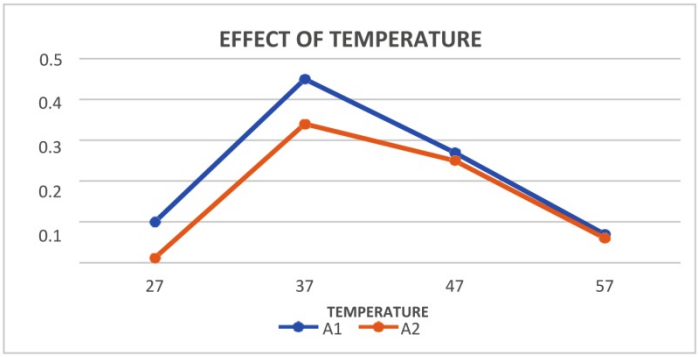


Fig 1 shows good pigment production at 37^{0}C

Pseudomonas aeruginosa and *Salinococcus roseus* showed good pigment production at 37^{o}C when compared to 47^{o}C and 57^{o}C. (Fig 1) and table 3.

Table 3.3 OD at different pH

Pigment	pH	OD at 550nm
A1	Control	0.0
	5	0.06
	7	0.25
	9	0.13
A2	Control	0.0
	5	0.07
	7	0.23
	9	0.16

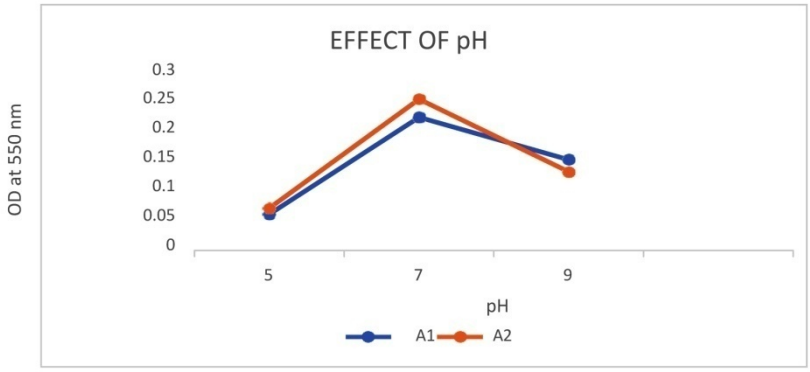


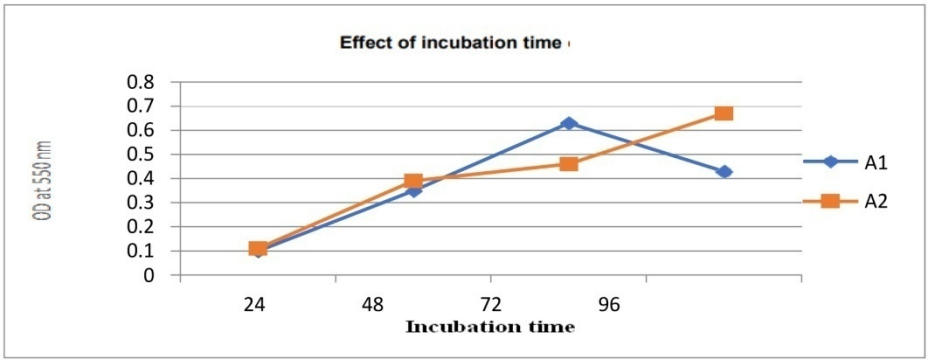
Fig. 2 Effect pH on pigment.

At pH 7 both isolates showed highest pigment production. (Fig 2 and table 3.3)

Table 3.4 shows incubation time of pigment producing bacteria

Pigment	Incubation time	OD at 550nm
A1	control24	0.0
	48	0.10
	72	0.35
	96	0.63
		0.43
A2	control24	0.0
	48	0.11
	72	0.39
	96	0.46
		0.67

Fig 4 shows effect of incubation time



Highest peak was observed at 96 hours of incubation for *Salinococcus roseus* and for *Pseudomonas aeruginosa* after 72 hours (Fig 4 and table 3.4). Difference in incubation time might be because of different generation time.

Static condition of A1 andA2

Using shaker A1 andA2

Figure 5 shows growth of isolates under shaker and static conditions

Pigment production elevated under shaker as shown in (Fig 5) than under normal static condition.

Figure 6 shows extraction of pigment

Pigment produced by isolates were extracted. The supernatant and pellet collected were kept before being further extracted using solvent. Ethanol was used for the pigment extraction. The extracted pigment were dissolved with solvent ethanol to evaluate antimicrobial activity against human pathogen by well diffusion method (Fig 6, 7, 8).

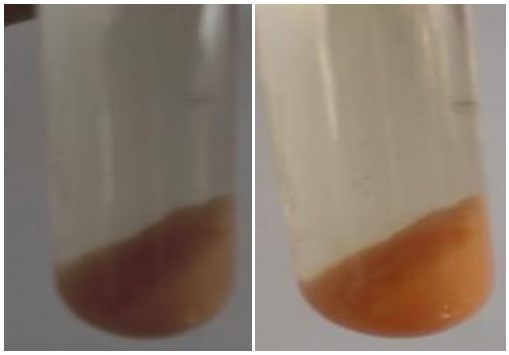


Fig 7 shows Pellet of the isolates $A1_I$ and$_I$A2

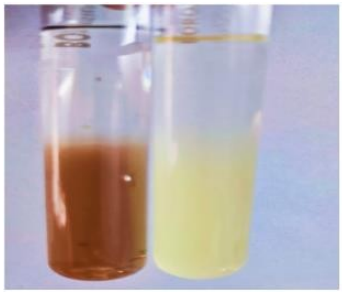


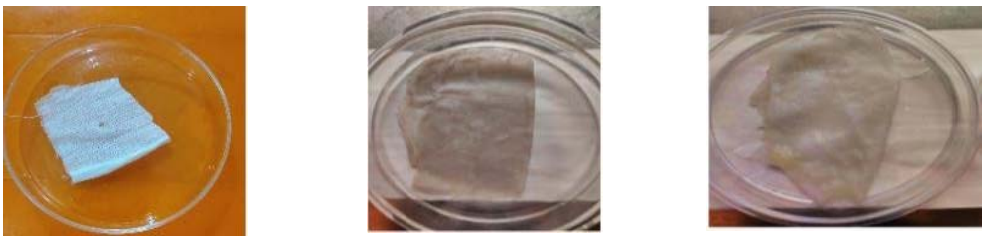
Fig 8 shows supernatant of isolate A1 and A2

Table 3.5 shows antimicrobial activity

Microorganism	A2	A1
E. coli	-	30 mm
Staphylococcus aureus	-	27 mm

Fig.9 shows anti-microbial activity of A1 and A2

The bacterial pathogens *E.coli* and *Staphylococcus aureus* were used. The zone of inhibition (table3.5) was measured to evaluate antimicrobial activity. *Pseudomonas aeruginosa* showed antimicrobial activity against these pathogens. No activity were seen in *Salinococcus roseus* isolate (Fig 9)



Control **A1** **A2**

Fig 10: shows application pigments on cloth

The extracted pigment tested for antibacterial activity and applied on cotton[17]. The cloth which absorb the pigment lightly were shown in (Fig 10).

3.2 Molecular Identification-

PCR amplification and sequencing of 16S rRNA gene sequencing.

The 16S rRNA gene was amplified by using primers. The gel image PCR amplified product of 16SrRNA gene. The PCR product were sequenced using universal primers. The obtained sequences were shown below.[18]

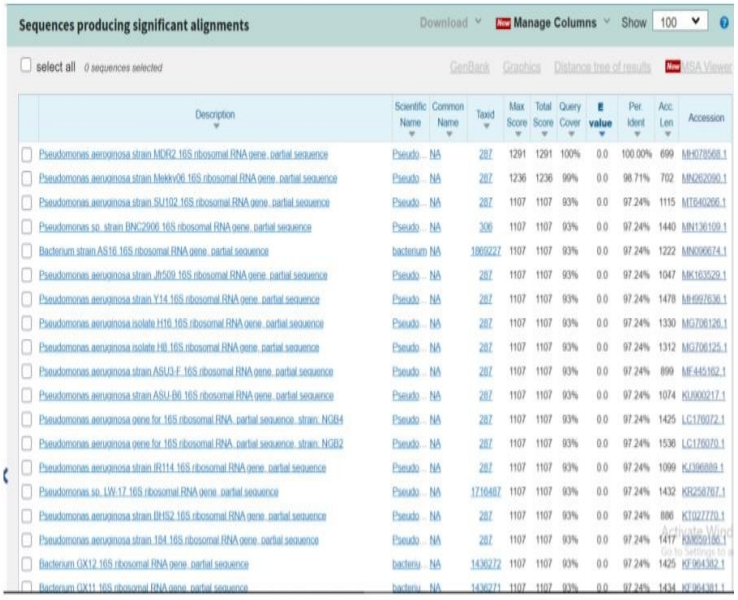
TACCATGCAGTCGAGCGGATGAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGG
GTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGG
CGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACG
CTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAA
GGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAATGAGACA
CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAA
GCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTT
TAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACA
GAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAA
GCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGGGGTTCAGCAAGTTGC
ATGTGAAATCTCCGGGCTCAACCTGGGAACTGCATCCACAACTACTGAGCTATAT
TACGGTAGATGCTGCTGGAATATCTTGTGGAGCGGCGAGGTGCGTCGACATCGG
ATGCAGCACGAGCGACCACGGTGTCTACTCGTGCTTGATTCTTCAAA

3.3 Similarity analysis

BLAST similarity search of NCBI shows 100% similarity with *Pseudomonas aeruginosa*

Figure 11 Graphical representation of sequence alignment of A1

3.3.2 BLAST similarity search result



Sequences producing significant alignments

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Description	Scientific Name	Common Name	Taxid	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Pseudomonas aeruginosa strain MDR2 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1291	1291	100%	0.0	100.00%	699	MH078568.1
Pseudomonas aeruginosa strain Mekkv06 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1236	1236	99%	0.0	98.71%	702	MN262090.1
Pseudomonas aeruginosa strain SU102 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	1115	MT640266.1
Pseudomonas sp. strain BNC2906 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	306	1107	1107	93%	0.0	97.24%	1440	MN136109.1
Bacterium strain AS16 16S ribosomal RNA gene, partial sequence	bacterium	NA	1869227	1107	1107	93%	0.0	97.24%	1222	MN096674.1
Pseudomonas aeruginosa strain JR509 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	1047	MK163529.1
Pseudomonas aeruginosa strain Y14 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	1478	MH997636.1
Pseudomonas aeruginosa isolate H16 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	1330	MG706126.1
Pseudomonas aeruginosa isolate H8 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	1312	MG706125.1
Pseudomonas aeruginosa strain ASU3-F 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	899	MF445162.1
Pseudomonas aeruginosa strain ASU-B6 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	1074	KU900217.1
Pseudomonas aeruginosa gene for 16S ribosomal RNA, partial sequence, strain: NGB4	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	1425	LC176072.1
Pseudomonas aeruginosa gene for 16S ribosomal RNA, partial sequence, strain: NGB2	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	1536	LC176070.1
Pseudomonas aeruginosa strain IR114 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	1099	KJ396889.1
Pseudomonas sp. LW-17 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	1716487	1107	1107	93%	0.0	97.24%	1432	KR258767.1
Pseudomonas aeruginosa strain BHS2 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	886	KT027770.1
Pseudomonas aeruginosa strain 184 16S ribosomal RNA gene, partial sequence	Pseudo...	NA	287	1107	1107	93%	0.0	97.24%	1417	KM659186.1
Bacterium GX12 16S ribosomal RNA gene, partial sequence	bacteriu...	NA	1436272	1107	1107	93%	0.0	97.24%	1425	KF964382.1
Bacterium GX11 16S ribosomal RNA gene, partial sequence	bacteriu...	NA	1436271	1107	1107	93%	0.0	97.24%	1434	KF964381.1

Sequence of 16S rRNA gene of the isolate A2

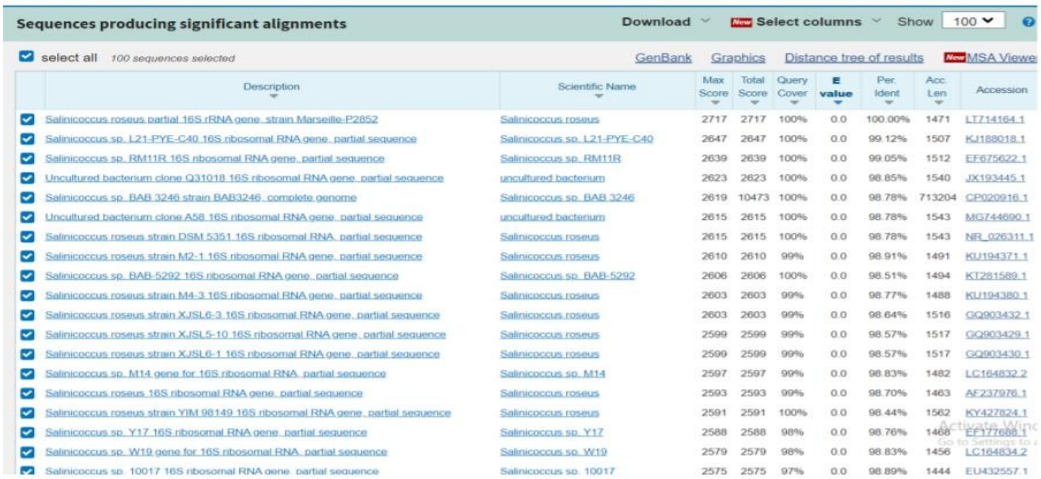
GATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGCGCGGATCAGGAGCTT
GCTCCTGTGACGCGAGTGGCGGACGGGTGAGTAACACGTAGGCAACCTGCCCACCAG
ACCGGGATAACCACGGGAAACCGTGGCTAATACCGGATAATCCTTCCCCACACAAGTG
GGGGAGTTGAAAGGCGGCTTCGGCTGTCACTGGTGGATGGGCCTGCGGCGCATTAGC
TGGTTGGTGGGGTAACGGCCCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTG
ATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGG
AATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGAAGAAGGGT
TTCGGCTCGTAAAACTCTGTTGTCAGGGAAGAACGCCGACGGGAGTAACTGCCCGTC
GGGTGACGGTACCTGACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAA
TACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTC
GTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGCG
AACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAG
ATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGT
GCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGAT
GAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCAC
TCCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGC

ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTT
GACATCCTCTGACCACCCTGGAGACAGGGTTTCCCTTCGGGGCAGAGTGACAGGTGG
TGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTTATCACTAGTTGCCAGCATTCAGTTGGGCACTCTAGTGAGACTGCCGGTGAC
AAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTAC
ACACGTGCTACAATGGACAGGAACAAAGGGCAGCTACGCCGCGAGGCCAAGCGAATC
CCATAAAACTGTTCTCAGTTCGGATTGGAGTCTGCAACTCGACTCCATGAAGCTGGAA
TCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGTCTTGTACACAC
CGCCCGTCACACCACGAAAGTCGGTAACACCTGAAGCCGGTGGGCCAACCTCTTGGA
GGCAGCCGTCGAAGGTGGGACCGATGATTGGGGTG

3.3.3 Similarity analysis

BLAST similarity search of NCBI shows 100% similarity with *Salinococcus roseus.*

Fig 12 graphical representation of sequence alignment of bacterium A2



Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Salinicoccus roseus partial 16S rRNA gene, strain Marseille-P2852	Salinicoccus roseus	2717	2717	100%	0.0	100.00%	1471	LT714164.1
Salinicoccus sp. L21-PYE-C40 16S ribosomal RNA gene, partial sequence	Salinicoccus sp. L21-PYE-C40	2647	2647	100%	0.0	99.12%	1507	KJ188018.1
Salinicoccus sp. RM11R 16S ribosomal RNA gene, partial sequence	Salinicoccus sp. RM11R	2639	2639	100%	0.0	99.05%	1512	EF675622.1
Uncultured bacterium clone Q31018 16S ribosomal RNA gene, partial sequence	uncultured bacterium	2623	2623	100%	0.0	98.85%	1540	JX193445.1
Salinicoccus sp. BAB 3246 strain BAB3246, complete genome	Salinicoccus sp. BAB 3246	2619	10473	100%	0.0	98.78%	713204	CP020916.1
Uncultured bacterium clone A58 16S ribosomal RNA gene, partial sequence	uncultured bacterium	2615	2615	100%	0.0	98.78%	1543	MG744690.1
Salinicoccus roseus strain DSM 5351 16S ribosomal RNA, partial sequence	Salinicoccus roseus	2615	2615	100%	0.0	98.78%	1543	NR_026311.1
Salinicoccus roseus strain M2-1 16S ribosomal RNA gene, partial sequence	Salinicoccus roseus	2610	2610	99%	0.0	98.91%	1491	KU194371.1
Salinicoccus sp. BAB-5292 16S ribosomal RNA gene, partial sequence	Salinicoccus sp. BAB-5292	2606	2606	100%	0.0	98.51%	1494	KT281589.1
Salinicoccus roseus strain M4-3 16S ribosomal RNA gene, partial sequence	Salinicoccus roseus	2603	2603	99%	0.0	98.77%	1488	KU194380.1
Salinicoccus roseus strain XJSL6-3 16S ribosomal RNA gene, partial sequence	Salinicoccus roseus	2603	2603	99%	0.0	98.64%	1516	GQ903432.1
Salinicoccus roseus strain XJSL5-10 16S ribosomal RNA gene, partial sequence	Salinicoccus roseus	2599	2599	99%	0.0	98.57%	1517	GQ903429.1
Salinicoccus roseus strain XJSL6-1 16S ribosomal RNA gene, partial sequence	Salinicoccus roseus	2599	2599	99%	0.0	98.57%	1517	GQ903430.1
Salinicoccus sp. M14 gene for 16S ribosomal RNA, partial sequence	Salinicoccus sp. M14	2597	2597	99%	0.0	98.83%	1482	LC164832.2
Salinicoccus roseus 16S ribosomal RNA gene, partial sequence	Salinicoccus roseus	2593	2593	99%	0.0	98.70%	1463	AF237976.1
Salinicoccus roseus strain YIM 98149 16S ribosomal RNA gene, partial sequence	Salinicoccus roseus	2591	2591	100%	0.0	98.44%	1562	KY427824.1
Salinicoccus sp. Y17 16S ribosomal RNA gene, partial sequence	Salinicoccus sp. Y17	2588	2588	98%	0.0	98.76%	1468	EF177688.1
Salinicoccus sp. W19 gene for 16S ribosomal RNA, partial sequence	Salinicoccus sp. W19	2579	2579	98%	0.0	98.83%	1456	LC164834.2
Salinicoccus sp. 10017 16S ribosomal RNA gene, partial sequence	Salinicoccus sp. 10017	2575	2575	97%	0.0	98.89%	1444	EU432557.1

Fig 13: shows sequence producing significant alignment.

Discussion

Many researchers identified several methods of pigment producing bacteria isolation. Soil and water microbial communities are among the most complex, diverse and important assemblages of organisms in the biosphere and they are an important source for the search of novel antimicrobial agents and molecules with biotechnological importance such as microbial pigments that can be used as natural colorants as well as antimicrobial agents in place of antibiotic [36]. The demand for new antibiotics continues to grow due to the emergency of antibiotics resistant pathogen causing life threatening infection in spite of considerable progress in the field of chemical synthesis and engineered biosynthesis of antimicrobial compounds [37]. Pigment producing bacteria were isolated on Nutrient agar media. Isolates with high pigment were considered for further studies. Two isolates A1 and A2 and were found to produce blue green and orange pigments. The pigment producing bacteria were identified based on their morphological, biochemical and molecular identification. Thus the selected A1 isolate showed 100% similarity with *Pseudomonas aeruginosa* and A2 showed 100% similarity with *Salinococcus roseus.*

Optimization studies done based on effect of pH, temperature, incubation time, shaking and static conditions. Temperature is one of the most critical environmental elements influencing microorganism growth, and it affects numerous metabolic pathways, including carotenoid production. *Pseudomonas aeruginosa* and "*Salinococcus roseus"* showed good pigment production at 37° C after 96 and 72 hours of incubation at a neutral pH. The difference in incubation time might be related to their different generation time. This also shows that the bacterium may be a moderate thermopile.

Maximum pigment was recorded at pH 7. Above pH 10 and below pH 5, no pigment production was recorded. The bacterial growth was observed up to pH 7 after which there is decline in

production was absorbed. Decline in growth and pigment production was observed from 72 hours in "*Salinococcus roseus"* and 96 hours in *Pseudomonas aeruginosa*, which suggest that microorganisms generally exhibit pigment production during late log phase or at stationary phase.

Salinicoccus roseus and *Pseudomonas aeruginosa* showed increased growth and pigment production at shaking incubation conditions as compared to static conditions. Microorganisms can improve transfer of substrates and oxygen in aerobic conditions.

Antimicrobial nature of isolate was done against two major bacteria *E. coli* and *Staphylococcus aureus*. Antimicrobial activities were exhibited by *Pseudomonas aeruginosa* alone. The pigments produced were extracted with ethanol and the extracted pigment was applied for dyeing. The results showed that the cloth can uptake the dye after 24 hrs of soaking [25]. From this we can conclude that *Salinicoccus roseus* and *Pseudomonas aeruginosa* may be used as viable source of environmentally friendly dyes and pigment with appropriate pH, temperature, and Incubation time. Also the antimicrobial activity of Pseudomonas strain can be explored more in the benefit of mankind.

Natural ingredients are seen to be viable source of environmentally friendly dyes and pigments. Despite substantial study in to bringing microbial pigments from the primordial soup to the market, their output will not be sufficient to meet demand if synthetic colors are phased out. Natural-color products may see a growth in demand soon, not only because of their health and environmental benefits but also because of their aesthetics and novelty.

Summary

Over all, this study focused on the isolation of pigment producing bacteria from the different sites. The selected bacteria were identified as *Pseudomonas aeruginosa and Salinococcus roseus* by 16S rRNA gene sequencing. Then various studies based on optimization studies, pigment production, extraction of pigment, antimicrobial activity of pigment and application of extracted pigment on cloth were done. Further studies can be focused on pure pigment production using column chromatography, and chemical structure analysis can be done by FT spectroscopy and commercial use can be explored.

Conflict of Interest: None

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