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RESEARCH ARTICLE OPEN ACCESS

Isolation and Molecular Characterization of Pigment Producing Bacteria from Soil of Different Locality of Assam

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Abstract

Pigment is the most attractive attribute and integral part of human life. The source of the pigment used for different applications is chemically synthesised. However, pigment produced from natural sources (such as plants and microorganisms) can also be applied as a coloring agent. In addition to being colorants, many natural pigments are also fascinating bioactive substances with possible health benefits. Agrochemicals, food, medicine, pharmacology, cosmetics, and numerous other industries use these compounds. In this study, a total of 7 pigmented colonies were isolated from the soil samples of different areas of Assam. Out of 7 pigmented colonies, 3 colonies showed orange color and 4 colonies showed yellow color. Based on the similarity of growth pattern and high intensity of pigmentation one colony from each pigment was picked for further studies. The isolated orange and yellow pigments were denoted as S1 and S2, respectively. With the help of Bergey's Manual of Determinative Bacteriology the isolated pigmented strains were preliminary identified based on their morphological, microscopic, and biochemical characteristics and after that 16S rRNA gene sequencing helped to identify the bacteria at the species level and the strains were identified as (S1) *Micrococcus aloeverae* ON377368 and (S2) *Exiguobacterium aestuarii* ON377409. The identified strains *Micrococcus aloeverae* and *Exiguobacterium aestuarii* showed a high pigment production rate at specific optimized conditions such as at temperature 37°C, pH 6, and NaCl concentrations at 1.5-2% and strains also showed a good characteristic growth pattern at a specific time interval. Therefore, the present study may be a helpful step towards the large-scale manufacture of pigments and the extraction, purification, and characterization of the pigment extracted from these strains will lead to provide a potent eco-friendly natural dye in the industrial sector.

Keywords: Natural, Pigment, Bioactive, Extraction, Strains

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INTRODUCTION

For human beings, color is one of the sources of attraction. It reflects the variety of geographical, cultural, sociological, and economic statuses among the population. In day-to-day life from every aspect, color influences human life. According to history from the ancient period, pigments have been used as coloring agents.[1] Pigments can be derived from both natural sources, such as plants and microorganisms, as well as synthetically produced compounds. However, synthetic pigments have several harmful effects on human health as well as the environment.[2] Knowing the negative effects of these synthetic coloring chemicals, people are becoming more conscious of them and become attracted to the harmless natural pigment. Natural pigments are mostly derived from plants, animals, or microorganisms. Many natural pigments are known to be fascinating bioactive substances with potential health effects in addition to being colorants. In addition to medicine, food, pharmacology, agrochemicals, and cosmetics, these compounds are also used in many other industries.[3] Bioactive pigments produced by microorganisms have been discovered in large quantities, and many of these pigments exhibit antioxidant, anti-inflammatory, and/or antibacterial properties.[4,5] During 2020 researchers predicted that due to the increased demand for natural products, the market for colorants developed at an estimated 7% annual pace and reached $7.79 billion in a year.[5,6] Many pigments have had their global markets for microbial pigments evaluated; for example, the carotenoids market was projected to reach $1.7 billion USD in 2020 and is predicted to grow at a rate of 2.6 percent to reach nearly $2 billion USD in the upcoming years. Astaxanthin, a type of carotenoid, had a market size of $192.5 million USD in 2020 and is expected to grow exponentially to approximately $228.4 million USD by 2027, according to AMR (2021) reports.[7,8] Therefore, the organics market and the pigment industries are sizable commercial sectors that will soon be dominated by microbial pigments.[5,9] The production of microbial pigments using fermentation technology is more dynamic and cost-effective than that of pigments derived from plants and animals. These pigments are biodegradable compounds with potential commercial uses as colorants.[3,10]

Fungal and bacterial microorganisms offer a convenient substitute source of naturally derived

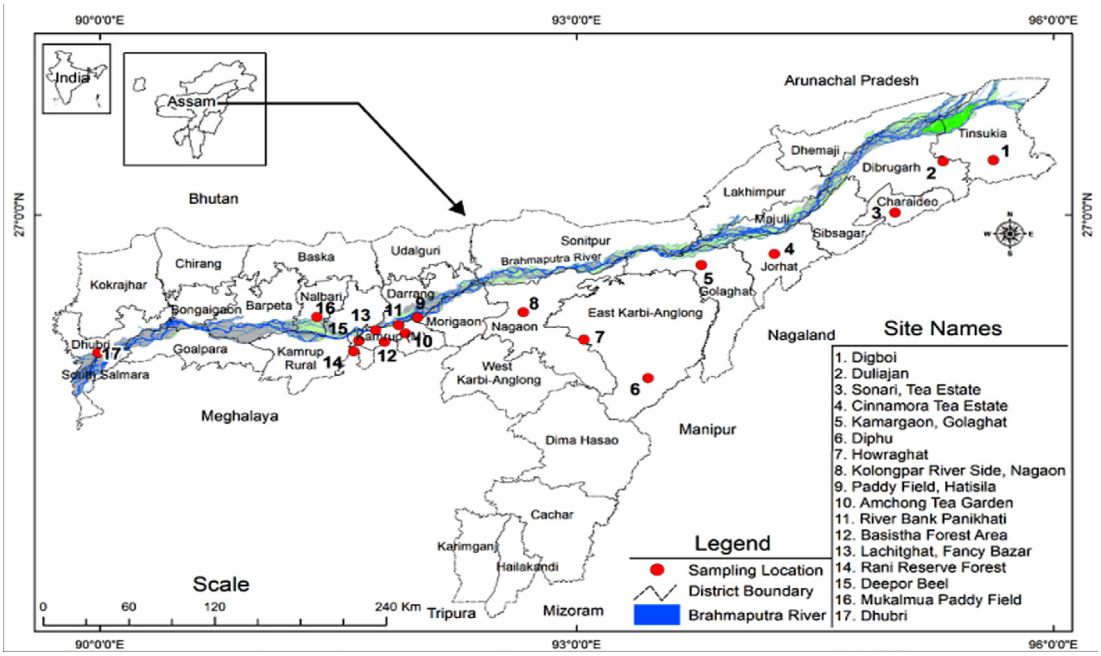

Figure 1. Map of Assam displaying the location of the sampling

pigments.[11,12] In comparison to fungi, bacteria are better at producing pigment because they have a shorter life cycle and are easier to genetically modify.[13] Most bacteria can produce several types of pigments such as astaxanthin, canthaxanthin, carotenoids, melanins, granadaene, indigoidine, flavins, and quinines.[11] According to the Munsell color system, most of the bacterial species produced a variety of coloring shades red (*Serratiamarcescens, Gordoniajacobaea*), red-yellow (*Kocuria* sp*., Chryseoacteriumartocarpi*), yellow (*Micrococcus, Hymenobacter* sp., and *Chryseobacterium* sp.), green (*Pseudomonas* sp.) blue (*Corynebacteriuminsidiosum, Erwiniachrysanthemi, VogesellaIndigofera*), purple (*Chromobacterium* sp.).[13] Many factors, including carbon, nitrogen sources, pH, temperature, time of incubation, moisture content, and aeration rate, affect the production of these bacterial pigments. Therefore, it is important to investigate their effects on the biosynthetic pathways that regulate pigment production in order to develop processes for increased production.[14] Considering these points, the present investigation was undertaken. The goal of the current study was to isolate and identify bacterial species that produce pigments in the natural environment, taking into account the diverse range of microbial pigments. In the current study, soil samples were screened for pigment-producing bacteria from different soil sample sites, including river banks, paddy fields, oil digging sites, tea gardens, etc.

METHODOLOGY

Collection of samples

For the investigation, soil samples were collected from several locations of Assam viz (Figure 1), Digboi, Duliajan, Sonari, Cinnamora, Golaghat, Diphu, Howraghat, Nagaon, Hatisila, Amchong Tea Garden, Panikhaiti, Basistha Forest area, Lachitghat, Fancy Bazar, Rani reserve forest, Deepor Beel, Mukalmua, Dhubri. According to the habitat of the soil, random sample locations were chosen from each area. The locations of the samples ranged from 25.50-27.23°" N and 89.59-95.37°" E (Table 1).[15] From sample sites, approximately 100g soil samples were collected at a depth of 0-20cm in a sterile plastic container, and after collection soil samples were stored at room temperature.

Table 1. Geographical features of the locations where the tested areas were collected throughout the distinct regions of Assam

No.	Place	Latitude	Longitude
1.	Digboi	27°23’17.99"N	95°37’16.78"E
2.	Duliajan	27°22’46.10"N	95°18’19.59"E
3.	Sonari, Tea Estate	27° 0’55.38"N	95° 0’6.78"E
4.	Cinnamon Tea Estate	26°43’16.31"N	94°14’32.22"E
5.	Kamargaon, Golaghat	26°38’32.93"N	93°46’57.50"E
6.	Diphu	25°50’22.95"N	93°26’50.91"E
7.	Howraghat	26° 6’44.16"N	93° 2’40.90"E
8.	Kolongpar River Side, Nagaon	26°18’24.82"N	92°39’44.69"E
9.	Paddy Field, Hatisila	26°16’10.61"N	91°59’49.37"E
10.	Amsang Tea Garden	26° 9’29.12"N	91°55’15.73"E
11.	River Bank Panikhati	26°12’56.25"N	91°52’40.24"E
12.	Basistha Forest Area	26° 5’52.15"N	91°47’25.30"E
13.	Lachitghat, Fancy Bazar	26°10’43.37"N	91°44’0.80"E
14.	Rani Reserve Forest	26° 1’47.09"N	91°35’47.30"E
15.	DeeporBeel	26° 6’21.55"N	91°37’41.95"E
16.	Mukalmua Paddy Field	26°16’23.76"N	91°21’47.53"E
17.	Dhubri	26° 1’6.82"N	89°59’8.30"E

Isolation and screening of pigmented bacteria

The collected soil samples were carried to the laboratory in a sterile container. Approximately 1.0g of materials were weighed, dissolved in 10mL of distilled water, serially diluted up to 10^{-8} dilutions, and then 0.1 ml of each dilution was plated over in a nutrient agar plate by using a sterile spreader. The plates were incubated at 35°C (±2) for 5 days and the plates at regular intervals for the appearance of the pigments (Figure 2). The pigmented colonies were screened and pure colonies of the strains were obtained by performing several rounds of the quadrant streaking method on sterile agar plates with 1.5% (w/v) of agar.[16] Pure isolated strains were sub-cultured on nutrient agar slant and plates and allowed to grow for 2-3 days at 37°C. After incubation, the plates and slants were kept in the refrigerator at 4°C for further study.

Morphological and biochemical characterization of pigmented isolates

With the aid of Bergey's Manual of Determinative Bacteriology,[17] the isolated pigmented strains were first identified based on their morphological, microscopic, and biochemical characteristics

Molecular characterization and Phylogenetic tree analysis of the isolated strains

Overnight-grown cultures of the isolated pigmented strains were used for DNA isolation. DNA was isolated by using a Macherey Nagel Nucleospin kit. For amplification of the 16S rRNA

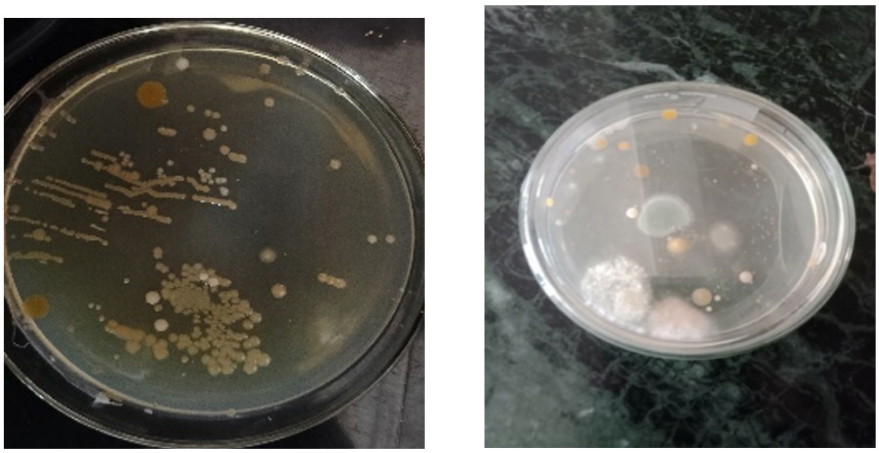

Figure 2. Isolation of pigmented bacteria

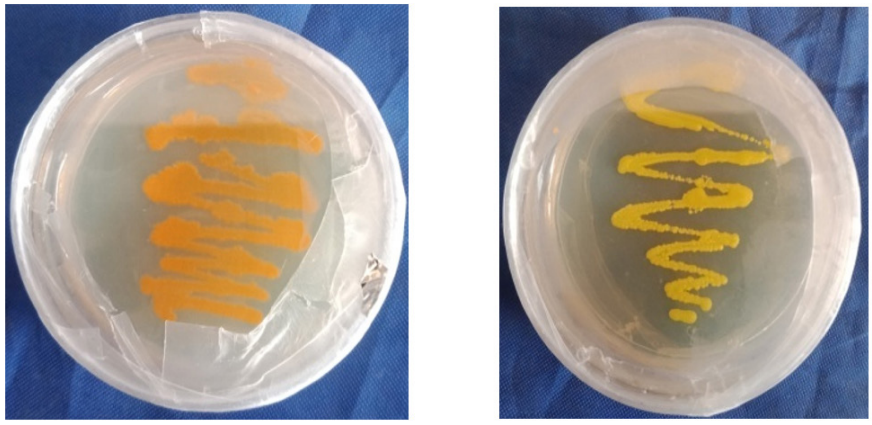

Figure 3. S1- Orange Isolates S2 Yellow Isolates

bacterial gene fragments, the 8F-806R (forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse primer 5'- GGACTACHVGGGTWTCTAAT-3') were used. For the PCR reaction, the optimized parameters were initial denaturation at 95°C for 5min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 54°C for 1min, extension at 72°C for 1min, and final extension at 72°C for 10 min. After amplification PCR products were electrophoresis by using 2% agarose gel. Amplified products were purified by using an ExoSAP-IT kit (Invitrogen) and were directly sequenced using an ABI PRISM BigDye Terminator V3.1 kit (Applied Biosystems, USA). The sequences were examined using the software Sequencing Analysis 5.2. The NCBI server's BlastN site (http://www.ncbi.nlm.nih.gov/BLAST) was used to perform the BLAST analysis. By adding the closest-matching reference sequences from the NCBI Genbank nucleotide sequence database, MEGA 11 software was used to create a phylogenetic tree for each of the sample sequences.

Table 2. Colony morphology of S1 and S2 isolates

	S1	S2
Colony Characteristics		
Colony shape	Round convex	Round
Colony sizes	0.5 mm	1-2 mm
Margin	Entire	Entire
Surface	Smooth	Smooth
Color	Orange	Yellow
Cell morphology		
Cell shape	Cocci	Bacilli
Gram staining	Gram-positive	Gram-positive

Growth curve of pigment-producing bacteria

The viable cells of the purified pigment-producing bacteria were inoculated into a sterile Nutrient broth. After that, the culture medium's incubation temperature was kept constant at 37°C under shaking conditions. The optical density of the bacterial growth at 405 nm (OD405) was measured for 72 hours at regular intervals of time to study the growth curve of the isolates.[18]

Optimization of different parameters for maximum production of pigment

For optimization of the maximum production of pigment, the following parameters were investigated: -

Table 3. Biochemical test for S1 and S2 isolates

Test	S1 Isolates	S2 Isolates
Catalase test	+ve	+ve
Indole	-ve	-ve
MR	+ve	+ve
VP	+ve	+ve
Citrate	+ve	+ve
TSI	+ve	-ve
Urease	-ve	+ve
Coagulase	-ve	-ve
Starch hydrolysis	+ve	-ve
Glucose	+ve	-ve
Sucrose	+ve	-ve
Maltose	+ve	-ve
Lactose	-ve	+ve
Mannitol	-ve	-ve
Arbinose	-ve	-ve
Xylose	-ve	-ve

Table 4. Specific Growth characteristics of the isolates

No.	Laboratory Code Designation	Species name and Accession Number	Specific growth rate (μm cm^{-3} min^{-1})	Generation time (h-1)	PCV / mL (' 10^6) After 24 Hrs	Colony texture in Nutrient agar media	Colony Colour in Nutrient agar media
1	S1	*Micrococcus aloeverae* ON377368	0.138 ± 0.032	7.14 ± 0.479	5.4 ± 0.75	Smooth, Opaque, Round	Orange
2	S2	*Exiguobacterium aestuarii* ON377409	0.145 ± 0.039	6.89 ± 0.441	3.9 ± 0.51	Smooth, Opaque, Round	Yellow

Effect of different temperatures on pigment production

The impact of incubation temperature on pigment production was examined by adjusting the NB medium's temperature to 25°C, 30°C, 37°C, 40°C, and 45°C. The Erlenmeyer flasks containing 100 ml of the fermentation medium (pH: 7) were inoculated with 10% of freshly prepared bacterial cell suspension and were incubated for 48-72 hours under shaking conditions of 200 rpm. After completion of the incubation period, the biomass yields (mg/ml) of the pigment-producing microorganisms were then determined as dry weight per volume. Also from the culture extracellular pigment was extracted by the methanol solvent separation method and The quantification of the pigment yields was done as per the formula.[19]

Pigment yield (µg/ml) = O.D520 x 17.072

Effect of pH on pigment production

The nutrient broth was prepared in different sets and before autoclaving the pH of the broth was adjusted by using 1M HCl and 1M NaOH to 2, 4, 6, 8, 10 respectively and inoculated with 10% bacterial cell suspension and incubated for 48-72 hours under shaking conditions to study the effect of pH for pigment production.

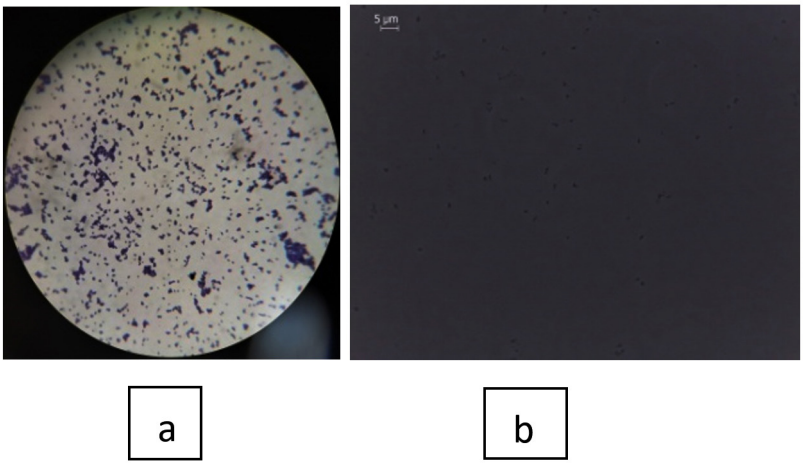

Figure 4(a, b). Gram staining and Phase contrast microscopy of S1 isolates

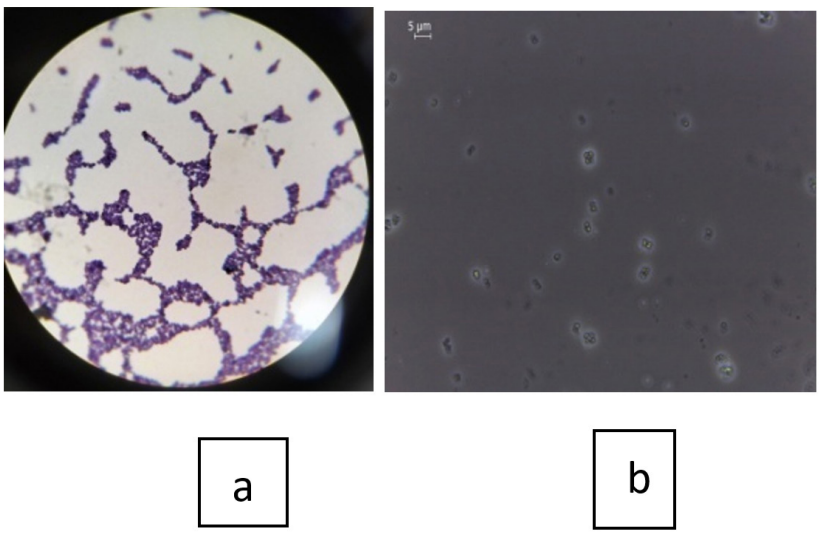

Figure 5(a, b). Gram staining and Phase contrast microscopy of S2 isolates

After completion of the incubation period similar methodology was adopted for biomass and pigment yield.

Effect of NaCl concentration on the pigment production

Nutrient broths were prepared in different sets and before autoclaving different NaCl concentrations were added (0.5%, 1%, 2%, 4%, 6%, 8%), respectively, and after that Erlenmeyer flask containing 100 ml of the medium was inoculated with 10% freshly prepared bacterial cell suspension and incubated for 48-72 hours to study the effect of salt concentration for pigment production. After the completion of the incubation period, a similar methodology which was applied to the effect of temperature conditions was adopted for biomass and pigment yield.

Statistical analysis

The experiments were carried out in triplicate, and the Analysis of Variance (ANOVA) test was used to statistically analyze the results.

RESULTS AND DISCUSSION

Isolation and Screening of pigment-producing bacteria

In this study, a total of 7 pigmented colonies were isolated from the soil samples (Table 2). Out of 7 pigmented colonies, 3 colonies showed orange color and 4 colonies showed yellow

Table 5. Effect of pH on pigment production

	Micrococcus aloeverae (S1)		*Exiguobacterium aestuarii* (S2)	
pH levels	Biomass yield	Pigment yield	Biomass yield	Pigment
2	3.4 ± 0.0192	8.2 ± 0.01581	3.7 ± 0.02702	8.4 ± 0.02074
4	4.0 ± 0.01581	9.8 ± 0.01817	4.0 ± 0.03847	10.6 ± 0.02739
6	7.9 ± 0.01483	**15.2 ± 0.03050**	8.2 ± 0.03033	**22.1 ± 0.03050**
8	3.6 ± 0.02074	8.1 ± 0.01924	3.9 ± 0.02864	9.9 ± 0.03782
10	2.2 ± 0.01140	6.0 ± 0.01393	2.4 ± 0.01673	5.3 ± 0.01924

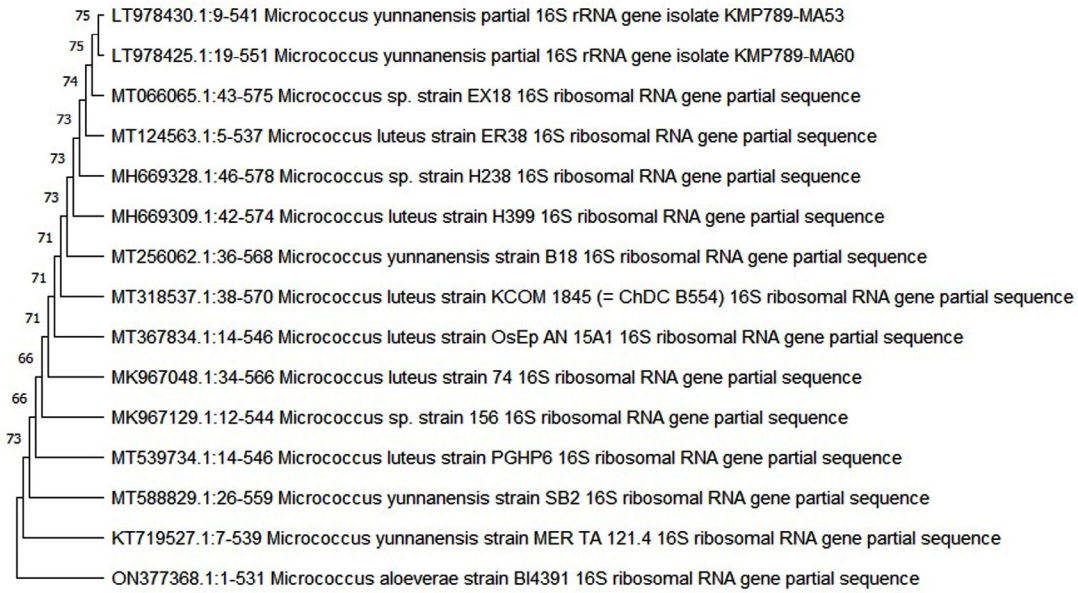

Figure 6. Phylogenetic tree of *Micrococcus aloeverae*

color. Based on the similarity of growth pattern and high intensity of pigmentation. One isolate from both pigments was selected for further studies. The isolate's orange colony was designated as S1 (Figure 3), and the yellow ones were designated as S2 (Figure 3).

Morphological and biochemical characterization of pigmented isolates

The isolated S1 and S2 strains showed good growth on the nutrient agar medium. According to the colony morphology and cell properties of the bacterial isolates on Nutrient agar, the isolate developed to form dense orange and yellow colonies that were 0.5-2 mm in diameter and had smooth consistency within 3 to 5 days of incubation.[20] The shape of the isolates and staining characteristics were identified by using Gram staining and biochemical analysis for both the isolates was analyzed by the Bergey Manual of Determinative Bacteriology and the results have been stated in Table 3 and 4. The Microscopic observation for both isolates were depicted in Figure 4 and 5. It was observed that similar biochemical results were carried out by the researchers Fatima and Anuradha, Prakash *et al*., and Arulselvi *et al.* in their study.[21-23]

Molecular characterization and phylogenetic tree analysis

The low range 100-1000 bp molecular marker was used to separate the PCR amplicons

Table 6. Effect of temperature on pigment production

	Micrococcus aloeverae (S1)		*Exiguobacterium aestuarii* (S2)	
Temp. (°C)	Biomass yield	Pigment yield	Biomass yield	Pigment yield
25°C	4.0 ± 0.01517	9.1 ± 0.02408	4.3 ± 0.01581	11.5 ± 0.03808
30°C	7.0 ± 0.01581	14.5 ± 0.02302	7.8 ± 0.04393	21.5 ± 0.03633
37°C	8.6 ± 0.02387	**20.0 ± 0.07266**	9.8 ± 0.02775	**27.2 ± 0.03033**
40°C	6.9 ± 0.01924	13.8 ± 0.02881	7.5 ± 0.11256	19.3 ± 0.04722
45°C	3.7 ± 0.02387	8.8 ± 0.03912	3.9 ± 0.05339	9.4 ± 0.04278

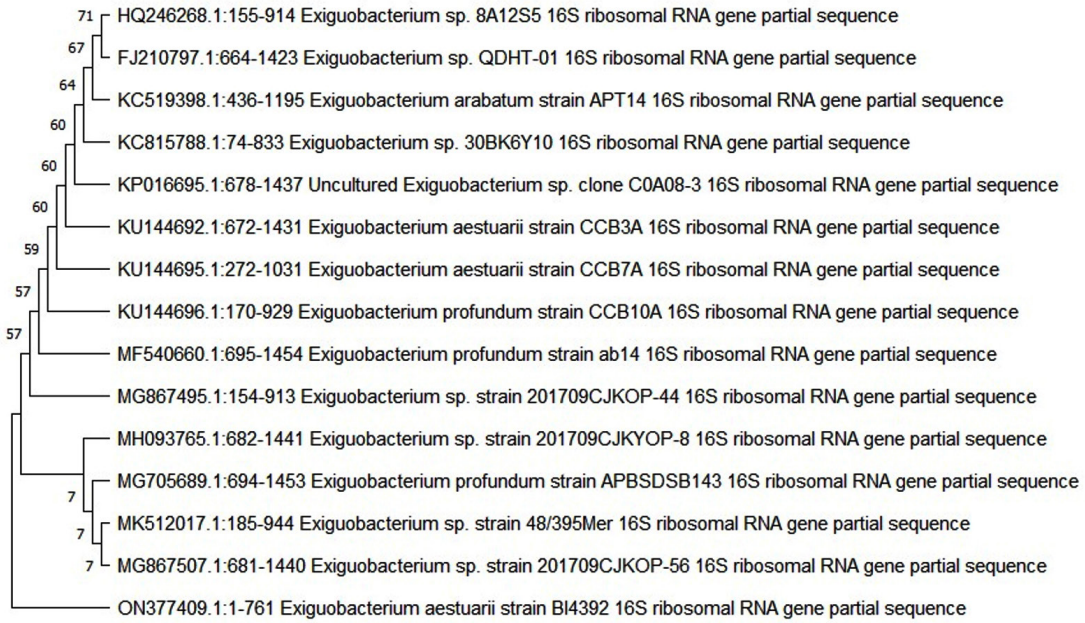

Figure 7. Phylogenetic tree of *Exiguobacterium aestuarii*

in agarose 2% gel, and the amplified products were recorded using the BIO-RAD GelDoc-XR gel documentation system. The obtained sequences were then compared to sequences in the Genbank nucleotide database. With 98-100% similarity, phylogenetic analysis is used to identify the species. Utilizing the help of MEGA11 software, phylogenetic tree analysis and sequence alignment was performed. Based on the similarity obtained from the database, the S1 isolate containing 531bp was identified as *Micrococcus aloeverae***,** and the S2 isolate containing 761bp was identified as *Exiguobacterium aestuarii.* The sequences were deposited in NCBI and the allocated accession no for *Micrococcus aloeverae* is ON377368 and *Exiguobacterium aestuarii* is ON377409 (Figure 6 and 7). The results revealed a high similarity of the 16S rRNA sequence of *Micrococcus aloeverae* and *Exiguobacterium aestuarii* with other species in the *Micrococcus* and *Exiguobacterium* genus, which was found by the different researcher in their study. The present result was in good agreement with Arulselvi *et al.*, Zhao *et al.*, and Shahin *et al.* Arulselvi et al. reported that the phenotypic characterization and 16SrDNA sequencing analysis helped to identify their two isolated yellow colour pigment-producing strains as *Exiguobacterium aurantiacum*.[22] In another study reported by Zhao *et al*. and Shahin *et al.,* they found two promising pigment-producing bacterial strain by using 16S rRNA sequencing analysis and they identified

Table 7. Effect of NaCl concentration on pigment production

	Micrococcus aloeverae (S1)		*Exiguobacterium aestuarii* (S2)	
Concen. of salt (%)	Biomass yield	Pigment yield	Biomass yield	Pigment yield
0.25	6.6 ± 0.02775	14.8 ± 0.01581	6.4 ± 0.02074	16.5 ± 0.30335
0.50	6.9 ± 0.01924	15.8 ± 0.01000	5.9 ± 0.04743	16.1 ± 0.15595
1.00	7.8 ± 0.03899	20.1 ± 0.05683	6.8 ± 0.03564	17.1 ± 0.03347
1.50	8.8 ± 0.05413	**24.0 ± 0.04970**	7.1 ± 0.01924	21.0 ± 0.03435
2.00	8.3 ± 0.01673	22.1 ± 0.01483	8.9 ± 0.01581	**25.4 ± 0.03194**

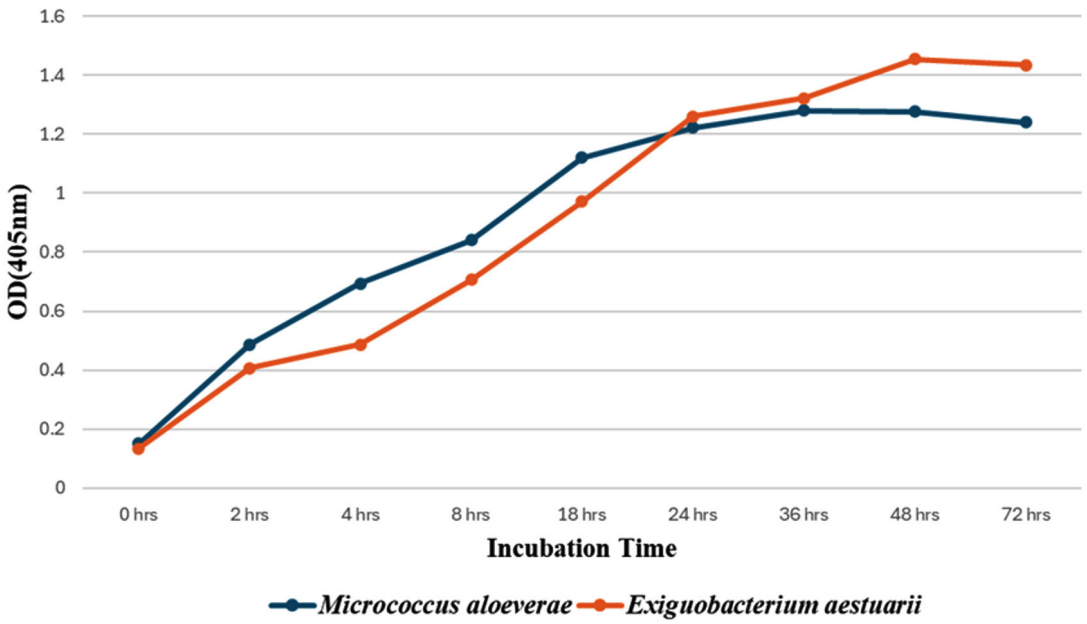

Figure 8. Growth curve of Micrococcus aloeverae and *Exiguobacterium aestuarii*

Table 8. Statistical analysis for biomass and pigment yield

Variables	Source of variation	Degrees of Freedom (DF)	Sum of square (SS)	Mean square	F- value	P- value
Biomass yields (pH) S1	Between Groups	92.969	4	23.242	83007.857	< 0.001
	Within Groups	0.006	20	0.000		
	Total	92.974	24			
Pigment yields (pH) S1	Between Groups	242.534	4	60.634	106374.572	< 0.001
	Within Groups	0.011	20	0.001		
	Total	242.545	24			
Biomass yields (pH) S2	Between Groups	96.149	4	24.037	28412.700	< 0.001
	Within Groups	0.017	20	0.001		
	Total	96.165	24			
Pigment yields (pH) S2	Between Groups	816.080	4	204.020	260895.005	< 0.001
	Within Groups	0.016	20	0.001		
	Total	816.095	24			
Biomass yields (NaCl) S1	Between Groups	17.841	4	4.460	3799.119	< 0.001
	Within Groups	0.023	20	0.001		
	Total	17.864	24			
Pigment yields (NaCl) S1	Between Groups	312.041	4	78.010	62209.199	< 0.001
	Within Groups	0.025	20	0.001		
	Total	312.066	24			
Biomass yields (NaCl) S2	Between Groups	25.036	4	6.259	6847.954	< 0.001
	Within Groups	0.018	20	0.001		
	Total	25.054	24			
Pigment yields (NaCl) S2	Between Groups	314.024	4	78.506	3280.383	< 0.001
	Within Groups	0.479	20	0.024		
	Total	314.503	24			
Biomass yields (Temperature) S1	Between Groups	89.103	4	22.276	41099.199	< 0.001
	Within Groups	0.011	20	0.001		
	Total	89.114	24			
Pigment yields (Temperature) S1	Between Groups	421.675	4	105.419	60239.249	< 0.001
	Within Groups	0.035	20	0.002		
	Total	421.710	24			
Biomass yields (Temperature) S2	Between Groups	124.831	4	31.208	8448.249	< 0.001
	Within Groups	0.074	20	0.004		
	Total	124.905	24			
Pigment yields (Temperature) S2	Between Groups	1074.274	4	268.568	173269.935	< 0.001
	Within Groups	0.031	20	0.002		
	Total	1074.305	24			

these strain as *Micrococcus yunnanensis* and *Micrococcus lyale*.[24,25]

Growth curve of pigment-producing bacteria

The growth curve of pigment-producing bacterial strains of *Micrococcus aloeverae* and *Exiguobacterium aestuarii* were studied to understand their growth pattern. The strain shows specific growth rate, generation time and Packed cell volume (PCV) in 72 hours of growth condition in the Nutrient broth and also in solid media shows smooth, opaque and round colony (Table 5). In the growth curve characteristic study, the stationary phase was observed between 24 and 72 hrs (Figure 8). in both the isolates. The above result of the present study is similar to the results found by the researchers Margino *et al.* for *Micrococcus* sp. and Park *et al.* for *Exiguobacterium* sp. study.[26,27]

Optimization of different parameters for maximum production of pigment

Effect of temperature, pH, and NaCl concentration on pigment production

Generally, the production of pigments by the bacterial strains depends on several factors. For the effect of pH and temperature on *Micrococcus aloeverae* and *Exiguobacterium aestuarii* the maximum pigment yield was obtained at pH 6.0 and the best temperature was 37°C (Table 6, 7 and 8). When the pH level increased, the pigment production rate decreased sharply due to the decrease in the growth of the bacterial cells. A similar study on pigment production was conducted by Choubey *et al.* for *Micrococcus* sp., Kandaswamy and Kathirvel for *Micrococcus luteus* and Fatima and Anuradha for *Exiguobacterium* and *Micrococcus* sp. where they reported that pH 7 and temperature 37°C was good optimized condition for pigment production.[21,28,29] According to Mohana *et al.*, *Micrococcus roseus* grew best at pH 8 and 37°C.[30] For the effect of NaCl concentration on *Micrococcus aloeverae* the maximum pigment yield was obtained at 1.50% NaCl concentrations and in the case of *Exiguobacterium aestuarii* the maximum pigment yield was obtained at 2% NaCl Concentrations. Other literature findings reported that the species belonging to the genus *Micrococcus* and *Exiguobacterium* showed the maximum pigment production at 2% and 4%, respectively.[21]

Statistical analysis

The post-hoc ANOVA analysis for biomass and pigment yields under different pH, NaCl concentrations, and temperature conditions are summarized in Table 8. The table presents the sources of variation (Between Groups and Within Groups), degrees of freedom (DF), sum of squares (SS), mean square (MS), F-value, and p-value for the respective factors and levels. In all the cases of this analysis was shown that the p-values are less than 0.001, indicating significant differences between groups. Additionally, the large F-values suggest strong evidence against the null hypothesis, indicating significant variability between groups compared to within groups. Therefore, the optimized conditions (pH, NaCl concentration, and temperature) significantly affect both biomass and pigment yields.

CONCLUSION

The result obtained from this study revealed that nature is the best habitat for microbial flora. Numbers of pigment-producing bacteria are present in the soil. In this study, the isolated pigment-producing bacteria were identified as *Micrococcus aloeverae* ON377368 (Orange) and *Exiguobacterium aestuarii* ON377409(Yellow). Both the isolates were collected from different soil habitats of Assam. These pigment-producing bacteria provide crucial information for the production of environmentally acceptable bio-color. Future research should concentrate on elucidating nature, and these pigments could be employed as a source of natural pigments for the food, pharmaceutical, and other cosmetic industries as well as for textiles.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Ethics Committee, Assam down town University, Guwahati, India, with the memo number AdtU/Ethics/Ph.D scholar/2021/056.

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