Article

Bacterial chromatics: exploring pigments and their practical applications

U. Sita ramyasri *, k. Naveena, ch. Jyothi ,
Department of microbiology, st. Anns college for women, mehdipatnam

*- corresponding author
Dr. U. Sita ramyasri
Assistant professor
Department of microbiology
St. Anns college for women mehdipatnam
Hyderabad, telangana.
Email: u.s.ramyasree@gmail.com

Abstract:

Background

Bacterial pigment production is a distinctive trait that can aid in bacterial identification and classification. These pigments not only contribute to the bacteria's visual appearance but also play vital roles in survival under extreme environmental conditions by acting as protective agents. Pigmented bacteria have found growing interest due to their potential applications in clinical diagnostics, biotechnology, and sustainable industries such as natural dye production and biomedical imaging.

Methods

Pigment-producing bacterial isolates including *micrococcus luteus* and *microbacterium oleivorans* were isolated and cultured under controlled laboratory conditions. Morphological characteristics were observed, followed by biochemical tests to determine metabolic and enzymatic properties. Pigment extraction was carried out using standard solvent extraction protocols, and the pigments were subjected to spectrophotometric analysis. The antibacterial and antioxidant properties of the pigments were also evaluated using agar well diffusion and dpph radical scavenging assays, respectively.

Results

The isolates exhibited distinct yellow and orange pigmentation under optimal growth conditions. Biochemical profiling confirmed the presence of catalase, oxidase, and amylase activities in the tested strains. Spectral analysis revealed pigments with absorbance peaks corresponding to carotenoid-like compounds. The extracted pigments showed moderate antibacterial activity against *staphylococcus aureus* and *escherichia coli*, and notable antioxidant potential, suggesting their application in pharmaceutical and nutraceutical industries.

Conclusion

Pigmented bacteria such as *micrococcus luteus* and *microbacterium oleivorans* demonstrate valuable biochemical and functional properties. Their pigments not only support bacterial survival but also present promising avenues for use in natural dyes, biomedical tools, and therapeutic agents. Further research into purification and structural analysis of these pigments could unlock novel applications in biotechnology and medicine.

Keywords: bacterial pigments, biocolors, eco-friendly, industrial application, pigmented bacteria, therapeutic application

Introduction:
Bacterial pigments are secondary metabolites produced by bacteria that help themselves adapt to various extreme conditions. Pigments are a characteristic feature of numerous bacteria. These pigments in the bacterial cell are capable to absorbing the uv rays and protect the cell. Bacterial pigments exhibit antimicrobial, antioxidant, anti-inflammatory, and anticancer properties. Hence these pigments can be extracted and used in various industries. Non-toxicity, biodegradability and non-carcinogenic properties of these pigments make them economically important. Bacterial pigments are colourful compounds produced by bacteria. The pigments can range in colour from yellow and orange to red, pink, purple, blue, green etc [1].

Few examples of bacterial pigments are carotenoids, anthocyanin, violacein, phycocyanin, prodigiosin etc. These pigments are known for their potential usage in food industry, drugs industry, as food colourants, dyes, paints etc. Colours are being added to various products for aesthetic purposes from the 1850's. The first synthetic colour 'mauvine' was developed by sir william henry perkin and from then on synthetic colours are used widely. Until recently when their consequences are observed [2].

Dyes such as tartrazine, cochineal red and sunset yellow are found to be provoking allergies when they react with other substances or on their own. Many of the synthetic pigments that are approved by the fda to be used as food colorants and in pharmaceuticals and cosmetics were later found to promote cancer. Benzidine dyes are proved to be causing bowel cancer. Carbon black which is widely used as printing ink pigment is highly carcinogenic. Observingr3 the human health vulnerabilities caused by the synthetic pigments, we ought to search for alternatives. Plants and microorganisms are the best alternative. In today's world, where the demand for natural colours has been increasing due to harmful effects of synthetic colours, bacterial pigments are a readily available alternative [3].
Microorganisms are known as potential source of bio pigment production due to their advantages over other natural colour sources like plants.

1. Microorganisms are readily available throughout the year unlike plants.
2. These pigments are cheaper and faster to produce.
3. Do not require much labour force.
4. These give higher yield with easy downstream processing.

These bacterial pigments extracted from microorganism are called bio colours. The popularity of these bio colours is increasing day by day due to the awareness regarding their safety and biodegradability. Out of all the microorganisms, bacterial pigments are more in use because of their short life cycle, high yield, variety of pigments produced by the bacteria [4].
Various pigments that can be observed in bacteria are:

Carotenoids:
Carotenoids are one of the most commonly found pigments in both plants and microorganisms. They are bright yellow-orangish colour which are lipid soluble. Carotenoids are known for their ability to keep the bacteria alive at low temperatures by modulating its membrane fluids. Carotenoids also absorb the uv rays to protect the cell.
Canthaxanthin is an orange-pink colour which is first isolated from *micrococcus roseus*. Echinenone is an orange carotenoid which is also isolated from the same bacteria. Numerous halophytic bacteria from the marine water produce astaxanthin which is a dark-red pigment. Staphyloxanthin is orange-membrane bound carotenoid found in *staphylococcus aureus* cells which imparts gold colour.

Carotenoids typically have a 40-carbon chain backbone compose of eight isoprene molecules. They are divided into carotenes containing only carbon and oxygen molecules. Carotenoid compound present in plants and bacterial cells have some similarities in their chemical composition but they are not identical compounds. Plant carotenoids like beta-carotene are produced by a complex pathway that occurs in plastids and other cell organelles. They are involved in photosynthesis and photoproductionand is responsible for the yellow colour in various fruits and vegetables. Whereas bacterial carotenoids are synthesized by the biosynthetic pathway that occurs in the cytoplasm. Bacterial carotenoids are involved in photoprotection and they also serve as anti-oxidants, pigments and signalling molecules [5].

Fig 1.1

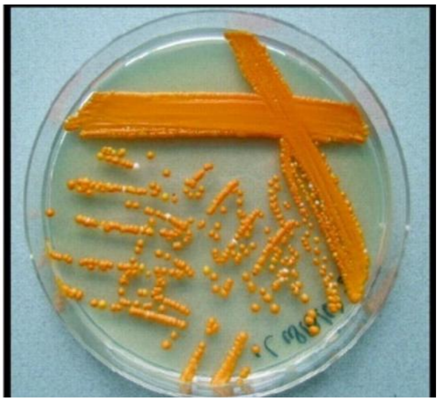


Fig 1.2

Fig 1.1 shows yellow-coloured bacteria and fig 1.2 shows orange-coloured bacteria representing the carotenoid pigment.

Prodigiosin:

Prodigiosin is a red pigment secondary metabolite first extracted from *serratia marcescens.* Prodigiosin is a linear tri pyrrole which is heat sensitive. Since prodigiosin is a large molecule made of several sub units, when exposed to high temperatures these sub units break down causing pigment to lose its characteristic colour. High temperatures can cause damage to the cells disrupting the biosynthetic pathway responsible for pigment production. Prodigiosin is a water insoluble pigment but soluble in other solvents like alcohol, methanol, acetone etc. Prodigiosin is usually found in gram negative bacteria like *pseudomonas magnesiorubra, serratia rubidaea* etc [6].

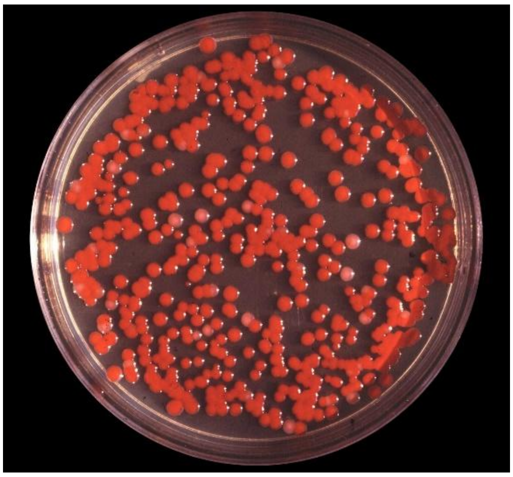


Fig 1.3

Fig 1.4

Fig 1.3 shows the red bacterial colonies and fig 1.4 shows the pink colonies whose pigment is identified to be prodigiosin.

Melanin:

Melanin is dark brown to black coloured pigments formed due to polymerized phenolic or indole compounds. Melanin pigment is also produced by humans in the hair, skin, eyes etc. Which protects from uv radiations.

Melanin is produced by a wide range of bacteria including bacillus, pseudomonas, and streptomyces. Bacterial melanin is synthesized from

Amino acids or aromatic compounds depending on the bacterial species. Bacterial melanin is used widely as a uv protectant and also in the treatment of certain diseases such as cancer and alzheimer’s disease.

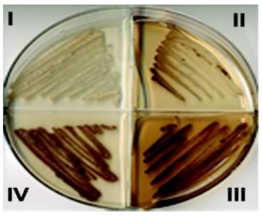


Fig 2

Fig 2 represents the different stages of growth of brown coloured bacteria whose pigment is called melanin. [7].

Violacein:

Violacein is a violet or purple coloured pigment found in *chromobacteriun violacein* first isolated from amazon river in brazil. The chemical structure of violacein consists of a central indole ring that is linked to two additional indole rings by methine bridges. The molecule also contains two amino groups and several oxygen atoms which contribute to its unique properties. Violacein pigment is found to have various properties like antibacterial, anti-oxidant, anticancer, and anti-inflammatory which makes it a potential source of usage in various industries [8].

Phycocyanin:

Phycocyaninis a pigment primarily found in photosynthetic organisms such as cyanobacteria. Cyanobacteria are a group of photosynthetic bacteria which are known for their ability to produce wide range of pigments. The chemical structure of phycocyanin is similar to that of other photosynthetic organism and consists of two protein subunits alpha and beta. These subunits are arranged in rod-shaped structure which is responsible for pigment’s distinctive blue colour.

fig 3 shows phycocyanin pigmented bacteria (green coloured bacteria)

Organism	Pigment	Color
Serratia marcesans	Prodigiosin	Red
Corynebacterium insidiosum	Indigoidine	Blue
Monascus roseus	Canthaxanthin	Orange, pink
Staphylococcus aureus	Zeaxanthin	Yellow
Pseudomonas aeruginosa	Pyocyanin blue	Green
Dunaliella salina	β carotene	Orange
Hematococcus pluvialis	Astaxanthin	Red
Bradyrhizobium sp.	Canthaxanthin	Orange /dark red
Alteromonas rubra	Prodigiosin	Red
Flavobacterium sp.	Zeaxanthin	Yellow
Asbhya gossypi	Riboflavin	Yellow
Blakeslea trispora	Lycopene	Rred
Rhodotorula sp.	Torularhodin	Orange-red
Penicillium oxalicum	Anthraquinone	Red
Streptoverticillium	Prodigiosin	Red
Streptomyces echinoruber	Rubrolone	Red
Janthinobacterium lividum	Violacein	Purple
Haloferax alexandrinus	Canthaxanthin	Dark Red

Table 1

table 1represents a table consisting of various microorganism and their pigments. We can see the diversity in the pigments produced by the bacteria and their colours.

Bacterial pigments have shown potential therapeutic applications due to their diverse biological activities, such as antioxidant, anti-inflammatory, anti-microbial, and anti-cancer properties. Some of the therapeutic applications of bacterial pigments are:

Fig 4 shows various coloured colonies

- Wound healing: bacterial pigments such as prodigiosin and violacein have been reported to promote wound healing by accelerating the process of tissue regeneration and reducing inflammation.
- Anti-cancer activity: several bacterial pigments have shown potential as anti-cancer agents, including prodigiosin, violacein, and pyocyanin. These pigments induce apoptosis (programmed cell death) in cancer cells, inhibit their proliferation and metastasis, and reduce the angiogenesis required for tumour growth
- Anti-inflammatory activity: bacterial pigments such as pyocyanin and indigoidine possess anti-inflammatory properties and can suppress the production of pro-inflammatory cytokines and chemokines.
- Anti-microbial activity: some bacterial pigments, such as prodigiosin and violacein, exhibit broad-spectrum antimicrobial activity against various pathogenic bacteria, fungi, and protozoa.
- Antioxidant activity: bacterial pigments such as carotenoids and phycobiliproteins possess antioxidant activity and can scavenge free radicals, protecting cells from oxidative damage.
- Anti-diabetic activity: bacterial pigments such as violacein have been reported to have anti-diabetic properties by improving insulin sensitivity and reducing blood glucose levels.

In conclusion, bacterial pigments have a wide range of potential therapeutic applications and are being extensively studied for their pharmacological properties. However, more research is required to fully understand the mechanisms of action and safety profiles of these pigments before they can be used as therapeutic agents in humans [9]. In addition to their ability to degrade pollutants, bacterial pigments can also be used to monitor environmental pollution. For example, some bacteria can produce pigments that change colour in response to changes in ph or the presence of specific pollutants, making them useful as biosensors for detecting environmental contaminants. Overall, the use of bacterial pigments in bioremediation shows promise as a eco-friendlier and more cost-effective alternative to traditional remediation methods. However, more research is needed to fully understand their potential and optimize their use in different bioremediation applications [10].

Methodology:

1. **Collection of samples:**

   Isolation of pigmented bacteria was done by *petri plate exposure method.* Nutrient agar plates are exposed to open air for 20 min. These plates are then incubated for 24-48 hours at 37 °c

2. **media used:**

   Nutrient agar media is used. Nutrient agar is a basic media that contains all the basic nutrients required for the growth of bacteria. Ph of this media is neutral (6.8).

3. **Isolation of pure cultures of pigmented bacteria:**specific coloured colonies were picked up from the exposed nutrient agar plates and streaked onto nutrient agar slants in sterile conditions and incubated at 37°c for 24 hours.

4. **Maintainence of pure cultures:**

   Pigmented bacteria grown on nutrient agar plates and slants are maintained at 2-20°c in the refrigerator and sub cultured into respective medium when required. These plates are preserved so that they can be used if needed.

5. **Characterization and identification of isolated pigmented bacteria:**

   Colony characterization for the isolated coloured colonies (on nutrient agar) was done based on size, shape, colour, opacity, margin, surface, elevation. These cultural characteristics are represented in a tabular form. Further characterization will be done by gram staining. The organism was further confirmed by 16srrna sequencing.

a. anti- bacterial activity:

Anti-bacterial activity of the various coloured bacteria is tested by *well diffusion method.* The test cultures used were*staphylococcus aureus* (gram positive) and *pseudomonas* (gram negative).

The test cultures were spread on muller-hinton agar (mha agar) using sterile swabs. Using a sterile tip, wells are made into the agar. 4 wells are dug on the muller hinton agar plate to check the degree of antibacterial activity between orange, pink, yellow and colourless bacteria.200µl of nutrient broth containing respective coloured bacteria is poured into each of the wells and incubated at 37°c for 24 hours.

5.3 **effect of temperature:**

Temperature can have a significant effect on the production of pigments in organisms. Temperature can affect the rate of pigment production. Higher temperatures can increase the rate of pigment production in some organisms while lower temperatures may slow it down. However too high or too low temperatures can be harmful to cells which may reduce or stop the pigment production altogether. To analyse the effect of temperature and also to find out the optimum temperature for the maximum pigment production in the orange-coloured bacteria this test is performed.

Take two sterile test tubes, with 5ml nutrient broth in each. Orange coloured bacteria is inoculated into both the tubes and incubated for 48 hours at 37°c and 27°c.after incubation, the amount of pigment production at different temperatures is measured using a colorimeter.

5.4 **Effect of ph:**

The production of pigments can be influenced by the ph of the growth medium, which affects the chemical properties of the environment that the bacteria lives in. Different bacteria have different ph optima, which is the ph at which the pigment is produced most efficiently. Take 3 test tubes with 5ml of nutrient broth in each. Equal amount of bacterial isolate is added to each one. To alter the ph, to one test tube add 1 ml of dil. Hcl and to the second test tube add 1ml of naoh and to the third test tube add 1ml of sterile distilled water. All of them are incubated at 37°c for 48 hours.using colorimeter, the amount of pigment production is measured to 540nm.

6 Change in pigment production on different media:

This is a test to check the pigment production of bacteria under various nutrient supplies and environmental conditions. To test this the pigmented bacteria are grown on different media. Orange, pink, yellow and red coloured bacteria are isolated and grown on nutrient agar media as pure cultures. These pure cultures are streaked on sabourads agar media under sterile condition. Then incubated at room temperature for 3-5 days.

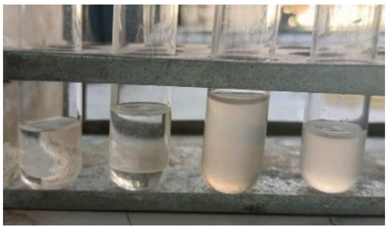


fig 5 shows that the bacterial pigment was diffused more into acetone than any other solvent.

7 **solubility test:**

Taking 4 sterile test tubes and 1ml acetone, ethanol, water and sodium acetate were added into different tubes. Equal amounts of pigmented bacterial culture are added to each of these tubes and left undisturbed for 1 hour. After 1 hour it is observed that the pigment is diffused more into acetone than any other solvent. Hence **acetone** should be used for the extraction process.

Extraction of the bacterial pigment:

Solvent extraction method is used for extracting the pigment. Scrape off the cells from the bacterial lawn culture using an inoculating loop and add into a centrifuge tube having 1ml of water. Shake the tube to make it homogenous. Then place it in centrifuge machine and centrifuge at 8000 rpm for 5-10 min. Remove the supernatant carefully without disturbing the pellet. Now to the tube add the solvent (tested and chosen after solubility test) acetone and heat it in water bath at 60°c for 15 min to break the bacterial cells. After cooling centrifuge again to remove the cell debris, collect the supernatant and to the pellet add acetone again and centrifuge at low speed again. Then line a funnel and filter the substance to remove any impurities. Wash once with ethanol to purify. Then let all the solvent evaporate to get the pigment.
Then scrape off the pigment to get it in powdered form to use it.
this process is used to extract orange-, yellow- and purple-coloured pigments.

6-results and discussions:
Isolation of bacterial pigment:

Pigmented colonies are isolated onto appropriate media after incubation

fig 6-plateshowing different pigmented colonies

Characterization and identification of isolated pigmented bacteria:

cultural characteristics of the isolated pure cultures were analysed morphologically based on several characteristics like shape, size, margin, surface, colour, etc.
The observations are shown below in table 2

Characters	Orange coloured bacteria	Yellow coloured bacteria
Shape	Circular	Circular
Size	Medium	Small
surface	Smooth	Smooth
Colour	Ornage	Yellow

.

Opacity	Opaque	Opaque
Elevation	Flat	Convex
Margin	Even	Even

table 2-showing colony morphology

1. Gram staining:

Gram staining was performed on the isolated pigmented bacteria to understand the chemical nature of the bacterial cell wall, structure and composition. Gram staining can help guide appropriate antibiotic therapy. The results are below in table 4.2.

table 4.2

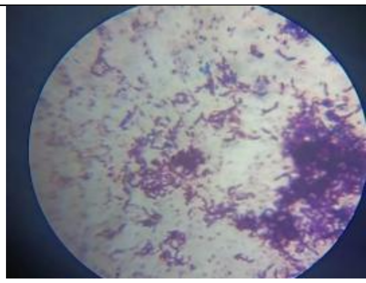
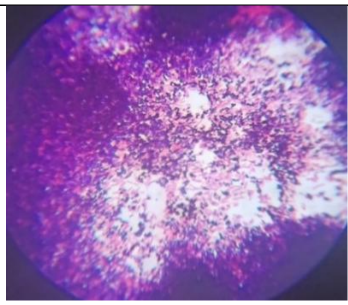
Orange coloured bacteria	Gram positive rods (chains)	
Yellowcoloured bacteria	Gram positive cocci	

table 3-gramstaining result

Description	Max Score	Total Score	Query Cover	E value	Per. Ident
Micrococcus luteus strain KCOM 1845 (= ChDC B554) 16S ribosomal R	1314	1314	99%	0.0	99.72%
Micrococcus luteus strain H399 16S ribosomal RNA gene, partial seque	1314	1314	99%	0.0	99.72%
Micrococcus sp. strain H238 16S ribosomal RNA gene, partial sequence	1314	1314	99%	0.0	99.72%
Micrococcus luteus strain MA3 16S ribosomal RNA gene, partial sequer	1314	1314	99%	0.0	99.72%
Micrococcus sp. strain EX18 16S ribosomal RNA gene, partial sequence	1314	1314	99%	0.0	99.72%
Micrococcus luteus strain 3F 16S ribosomal RNA gene, partial sequence	1314	1314	99%	0.0	99.72%
Micrococcus luteus strain NCCP 16831 chromosome, complete genome	1314	2606	99%	0.0	99.72%
Micrococcus sp. strain MA_AMC_56 16S ribosomal RNA gene, partial s	1314	1314	99%	0.0	99.72%
Micrococcus sp. strain SCA_CLR_217 16S ribosomal RNA gene, partia	1314	1314	99%	0.0	99.72%
Micrococcus sp. strain DSM 105846 16S ribosomal RNA gene, partial s	1314	1314	99%	0.0	99.72%

From 16srrna sequencing the above organism was identified as *micrococcus luteus*

Description	Max Score	Total Score	Query Cover	E value	Per. Ident
Microbacterium sp. strain RYA6 16S ribosomal RNA gene, partial sequen	1598	1598	100%	0.0	99.66%
Microbacterium sp. strain EB193 16S ribosomal RNA gene, partial seque	1598	1598	100%	0.0	99.66%
Microbacterium oleivorans strain gene 16S ribosomal RNA gene, partial s	1598	1598	100%	0.0	99.66%
Microbacterium sp. strain NQS17 16S ribosomal RNA gene, partial sequ	1598	1598	100%	0.0	99.66%
Microbacterium sp. strain S9-TSA-14 16S ribosomal RNA gene, partial se	1598	1598	100%	0.0	99.66%
Microbacterium oleivorans strain MV19 16S ribosomal RNA gene, partial	1598	1598	100%	0.0	99.66%
Microbacterium oleivorans strain MV1 16S ribosomal RNA gene, partial s	1598	1598	100%	0.0	99.66%
Microbacterium oleivorans strain A9 chromosome, complete genome	1598	3196	100%	0.0	99.66%
Microbacterium sp. strain LA19P1 16S ribosomal RNA gene, partial sequ	1598	1598	100%	0.0	99.66%
Microbacterium sp. strain LA16P37 16S ribosomal RNA gene, partial seq	1598	1598	100%	0.0	99.66%

The organism was identified as *microbacterium oleivorans*

2. Anti-bacterial activity:

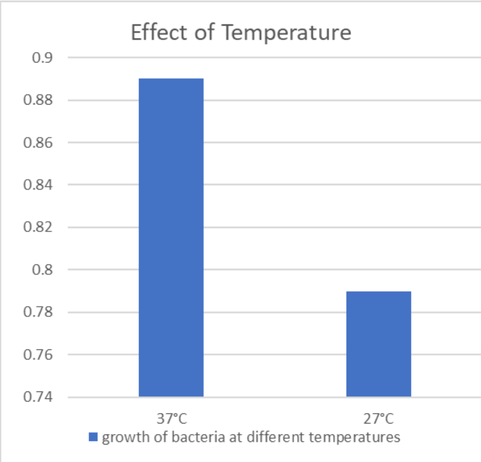
- No significant activity was shown by any of the bacteria against pseudomonas.
- Orange coloured bacteria showed slight zone of inhibition against staphylococcus aureus of about 3mm. No significant activity is seen from other bacteria.

Fig 4.4

Fig 7 shows the slight zone of inhibition shown by orange-coloured bacteria against s. Aureus.

Pseudomonas species are known to be highly resistant to a wide range of antibiotics and other antibacterial compounds. None of the pigmented bacteria were able to show anti-bacterial effect against pseudomonas. This can be due to, either the antibacterial compounds produced by pigmented bacteria are not effective against pseudomonas or the concentration of the antibacterial compound is too low to show effect. For staphylococcus aureus, orange-coloured bacteria were able to show slight zones of inhibition.

1. **Effect of temperature:**



After incubation, the range of pigment production from both isolates was checked using colorimeter technique at 540nm.pigment production is more at 37°c. We can also conclude that pigment production alters with temperature.from this test we can conclude that the temperature optima of the orange-coloured bacterium are 37°c.the enzymatic activity, metabolic activity and the pigment production were higher at 37°c. The y-axis of the graph indicates the values of optical density and the x-axis indicates the different temperatures in which the bacteria are

Fig 8.1 clearly shows the difference between the production of pigment at 37°C and 27°C.

grown.

2. **Effect of ph:**

After incubation the amount of pigment production is measured using colorimeter technique.

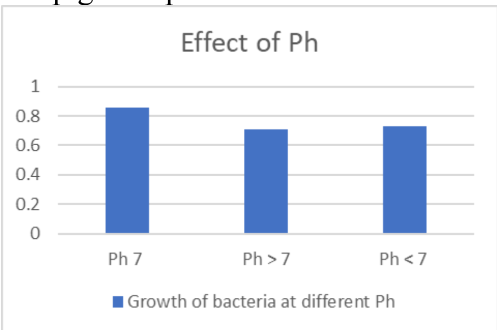


Fig8.2-showing effect of ph

We can conclude that pigment production is maximum at neutral ph 7 and when ph is altered the pigment production is also altered. Pigment production was lowered when the ph is altered. It is possible that the enzymes involved in pigment production have an optimum activity at ph 7, hence more pigment production.

3. **Extraction of the bacterial pigment:**

Solvent extraction method was performed to extract the bacterial pigment by using acetone as the solvent. Once the solvent in the supernatant collected is evaporated, the pigment becomes dry and powdery in form. Scrape it off using a spatula and collect it carefully. These pigments are extracted in larger amounts to be used in various industries as food colorants, dyes, in cosmetics etc. Purification methods of these pigments are very essential to make sure no cell debris or any impurity is present in this pigment. We must make sure that this is the pure form of bacterial pigment before usage. All the tests and analysis performed before help us identify what type of compounds need to be used at each and every step of processing.

4. Application:

The bacterial pigment that is extracted is used to create a few products so that we can understand what they are capable of. These pigments are used in two applications. They are as follows.

1. **Candles:** candles can be made into colourful ones by the addition of this pigment. Candle wax is heated to turn into a liquid and then the pigment is added, mixed and then allowed to cool down. Once it turns into a solid candle the results can be evidently seen.

fig 8.1 shows different pigments extracted are added to candles.

Paints: for the paints, generous amount of water is added to turn it into a water colour which can be used to make paintings. Water colours can also be made from these pigments. These pigments are used to make a painting which was also bright and colourful. The difference in the before and after images of the products are enough to explain the potential of these bacterial pigments. These pigments when added to candles serve as a therapeutic application. These candles when lit up spread many substances in air which are capable to inhibit harmful microorganisms. These candles stand ahead from all the other candles in the market because they are not just colourful and vibrant but also removes impurities in air.if these pigments are added to soaps, they too will act as a powerful agent to remove harmful pathogens on your body. Not just candles and water colours, but these pigments can also be added to soaps, clothes, any variety of paper, etc.

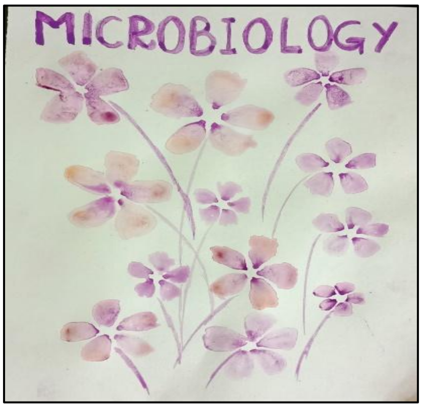


fig 8.2 showing pigments used as paints or colours to create a drawing or painting.

7-conclusions:

Bacterial pigments are an emerging field of study with a wide range of potential applications in various industries. These pigments offer natural and sustainable alternatives to synthetic dyes and have been shown to possess various beneficial properties, including antimicrobial, antiviral, and anticancer activity. Additionally, bacterial pigments can contribute to sustainability efforts through their use of renewable resources and waste reduction. Recent advancements in bioproduction, genetic engineering, and functionalization have opened up new possibilities for the development and optimization of bacterial pigments for various applications. However, more research is needed to fully understand their safety, efficacy, and environmental impact, particularly in the context of commercial production and release of genetically modified bacteria. Overall, the study of bacterial pigments offers promising possibilities for the development of natural and sustainable solutions to various challenges in industries such as healthcare, agriculture, and environmental sustainability.

8-conflict of interest

None

9-funding

None

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