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ISOLATION AND IDENTIFICATION OF BACTERIA PRODUCING PIGMENTS FROM GARBAGE DUMPED GROUND SOIL

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Abstract

In this present study the pigment producing microorganisms were isolated from garbage dumping ground and identified its morphological and biochemical characterization. The bacterial isolates were *pseudomonas sp*, *serratia sp*, and *staphylococcus sp*. These isolates were produced the coloured pigments. In this study much focus is on the production of natural pigment from bacterias. The pigment producing bacteria were isolated from garbage dumping ground and the isolate are *Serratia sp, Pseudomonas sp, Staphylococcus sp.* This isolates was produce various coloured pigments such as green, yellow, orange. These pigments were extracted from the broth by centrifugation method. These extracted pigments shows antimicrobial activity against human pathogen such as *E.coli.* Then these extracted pigments were applied on the textile industry. The use of synthetic dyes in these industry cause problems in environment such as toxicities. The synthetic dyes are not environment friendly. The natural colours are more advantage than synthetic colours, they have antibacterial, antifungal, viricidal, anti-oxidant, anticancer activities etc.

Key words:- Pigment, *Pseudomonas sp, Serratia sp, Staphylococcus sp,* extraction and antimicrobia activity.

Introduction

Colours are the most pleasing and first parameter to be noticed about any article by the receptor. Colour provides attracting appearance to marketable products such as Food Products, textiles, and pharmaceutical products. Colours are the beauty of world that means we cannot imagine world without colours. Colours is an integral part of both human culture and in human life colours have been used to enhance the aesthetic value of every day human life.[1]

Plant and micro Organisms are two major sources of natural pigments. Yet the natural pigments from plants also have drawbacks such as instability against light, heat or adverse pH, low water stability and are often non availability throughout the year hence the microbial pigment are of great interest owing to the stability of the pigments produced and the availability of cultivation technology. Microbial Pigments have many advantages over artificial and inorganic colour.[2]

The pigment production from Microorganisms is efficient and beneficial process as compared to chemical synthesis of pigments. Microbial colours are available in different shades. These colours are biodegradable and environment friendly. They also have numerous clinical charter restive like antioxidant, anticancer, antiproliferative, immunosuppressive, treatment of diabetes mellitus etc. Microbial pigment productions is now one of the emerging fields of research to demonstrate its potential for various industrial applications.[3]

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Bacterial pigments have many applications in current day life. Beside bacteria, Fungi, yeasts, plants and other natural sources produce pigments nature is rich in colour obtained from, fruits, Vegetable, roots, minerals, plants, microalgae. As they originate from biological material they are often called 'biocolours'.[4]

Pigments of various colours are synthesized to protect the microorganisms from injurious effect of light rays of visible and near UV range .The microorganisms which have the ability to produce pigments in high yield included species of , Monascus, Pseudomonas, serratia, cordyceps, streptomyces, Xanthaphyllomyces, Saccharomyces, Rhodotorcila, Mucor, Ashbyagossypi, staphylococcus aureus , Cyanobacteria. Microorganisms produce various pigments like Melanin, Astaxanthin, pyocyanin, prodigiosin, Torularhodin, B.Carotene, Monascorubramin, Riboflavin, Violacein, Staphyloxanthin.[5]

Microorganisms plays a vital role in the pigment production. Microorganisms are a promising source of natural colours. The microorganisms are including in the pigment production, Bacteria, Fungi, Yeast, algae and protozoa. These Microorganisms can be isolated, cultured, purified from environmental sources. Such as water bodies, soil, plants, insects and animals; Mainly from water bodies and Soil.[6]

Microorganisms which have the ability to produce pigments in high yields include species of *Monascus, paecilomyces, Serratia, Cordyceps, Pseudemonas, Staphylococcus,Streptomycess, Bacillus sp, Cyanobacteria* etc.[7]

Photosynthesis is a major reason for bacterial pigment production (Chlorophylls, Carotenoids) eg. green and purple sulfur bacteria. The other reason are UV protection defense mechanisms, secondary metabolites for storage of energy and stress. Bacteria produce colourful or colourless, Molecules for their own benefits. Most of the microorganisms to produce pigments are not soluble in water. For example is *serratia sp* insoluble in water. It is soluble in some solvents like methanol, ethanol, chloroform etc. *Sarretia sp* is reported to produce red colour pigment.[8]

Human have always preferred natural sources for colourings of food, clothing cosmetic and medicines. A variety of natural and synthetic pigment are available naturally derived pigments are represented by carotenoids, flavonoids.[9]

Carotenoids are the most widely observed pigments. These pigments have some important function such as they act as the protective agents against some oxidative damage. These carotenoids have to increase tolerance to ultra violet radiation. This means pigment have different function, pigment molecules have flexible abilities to be at several energetic level changing the corresponding colour, than for the primary functions are the energy storage, transfer and Filter other functions seem to be secondary in evolution from the ocean.[10]

Factors affecting Microbial Pigment production such as, Temperature, pH, carbon sources, minerals, moisture content, aeration rate can be affected the yield. Application of bacterial pigments produced by bacterias were traditional use in oriental countries and have been a subject of intense research in the present decades because of its potential for applications like, textile industry, food industry, pharmaceutical industry, etc. The aim of the study is to isolate and identify the bacteria produce pigments from garbage dumping ground soil.

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In this present study the production of Bacterial pigments from Grabage dumping ground soil have rich source of microorganisms, they also produce colours for various application. The synthetic colour are not environment friendly. Nowadays the demands of natural pigments re very high. The collected soil samples contain different type of microorganisms mainly bacteria. These are produce microbial pigments. The various coloured pigments are extracted by different ways like centrifugation, Filtration, etc. The extracted pigment can be applied into various industries mainly textile, food, pharmaceuticals etc.

Materials and Methods

Collection of sample

The soil samples were collected from garbage dumping ground near Parvathipuram, Nagercoil, Kanyakumari district, Tamilnadu. The pigment producing bacteria was isolated from these samples.

Isolation of pigment producing organism

The collected soil sample was aseptically serial diluted up to 10^{-4} to 10^{-9} dilutions with sterile water. 1 ml of each dilutions are inoculated on sterile nutrient agar plate. The growth on the plates were observed after 24-48 hrs of incubation .The pigment producing bacterial colonies alone were selected and subcultured.

Primary screening of pigment producing microorganism

The isolated colonies were picked and cultured separately on their respective growth media at specified growth condition for individual pure cultures. One loopfull of isolated pigmented colonies was quatrantly streaked on citrimide agar plate for bright pigmentation. One loopfull of other pigmented colonies were streaked on nutrient agar media. These were awaited for 2-4 days for bright coloured colonies. The coloured colonies were picked and subcultured for further experimentation.

Characterization and Identification of Pigment Producing Bacteria

The various types of morphological and biochemical tests were carried out for the identification according to Bergey's Manual of Determinative bacteriology.

Morphological characterization

The isolated colonies on the basis of size, shape, colour, opacity, consistency, elevation, margin etc. In the current study were performed two basic morphological characterization such as Gram's staining and motility test.

Biochemical characterization

In the present work were characterized by Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test, Catalase test and Carbohydrate fermentation test were performed. In this present study the pigment producing microorganisms were isolated from garbage dumping ground and identified its morphological and biochemical characterization. The bacterial isolates were *pseudomonas sp*, *serratia sp*, and *staphylococcus sp*. These isolates were produced the coloured pigments .The results were compared with previous studies.

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Samyuktha and Sayali, (2016) worked on pigments produced from *serratia sp* ,for the isolation of prodigiosin pigment.[11] In this study Nutrient agar medium can be used for the isolation of pigment producing bacteria and pigment production. In our study centrifugation and filtration methods were used for the extraction process. Pigment was extracted using methanol as organic solvents. The pigment showed antimicrobial activity against *E.coli*, *S.aureus*, *klebsiellia* etc.

Screening of the pigment producing bacteria

The isolated colonies of identified pigmented cultures were inoculated in to 100ml sterile Nutrient broth in conical flask. Conical flasks were incubated of rotary shaker for 24-48 hrs. The extensive growth of pigment producing bacteria was seen in the flasks after 48 hrs of incubation.

Extraction of pigments from pigment producing bacteria

After incubation pigment producing bacteria was extracted by centrifugation method. The pigmented broth were centrifuged at 6000rpm for 15 mins.

Method -1

After centrifugation the supernatants were collected and pellets were discarded. The collected supernatants were mixed with equal amount of methanol solvent and filtered through whatman filter paper.

Method -2

After centrifugation the supernatants were discarded and the pellets were re-suspended in methanol. Then the mixture was vortexed and the suspension was centrifuged at 6000 rpm for 10 mins and the supernatant was collected .Centrifugation was repeated till the pellets change to colourless. After centrifugation the supernatant was filtered through whatman filter paper.

Antimicrobial activity

Antimicrobial activity of the extracted pigment was checked by using well diffusion method. The human pathogens (*E.coli*) was used against extracted pigment to evaluate its antimicrobial activity. The pathogen was inoculated on to sterile nutrient agar plate by spread plate method. Then the well was bored in the plates . The wells were filled with appropriate amount of pigment. Then the plates were incubated at 37^0C for 24-48 hrs. After incubation the result was observed by measuring zone of inhibition.

Application of bacterial pigment in textile industry

A pre washed cotton cloth was immersed in 0.5% Nacl solution for 10 mins for fixation. After fixation the cloth was placed on the extracted pigment to absorbing the colour. The cloth was kept for 4-5 days.

Result and Discussion

In the present study, the soil samples were collected from garbage dumping ground near Parvathipuram, Nagercoil, Kanyakumari district, Tamilnadu. The pigment producing bacteria was isolated from these samples (Figure: 1 and 2).

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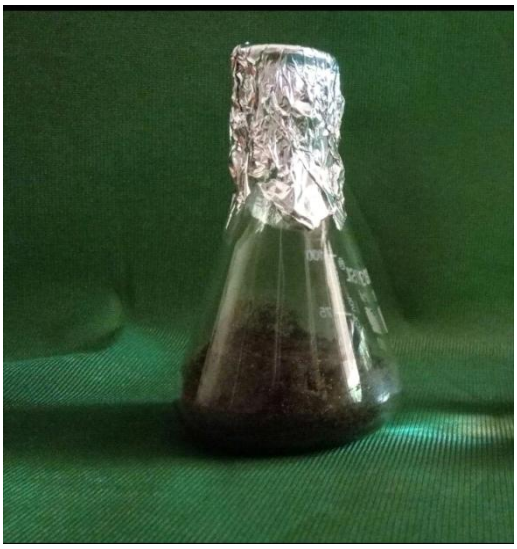
Figure 1: Sample Collection Site

Figure 2: Collected Soil Sample



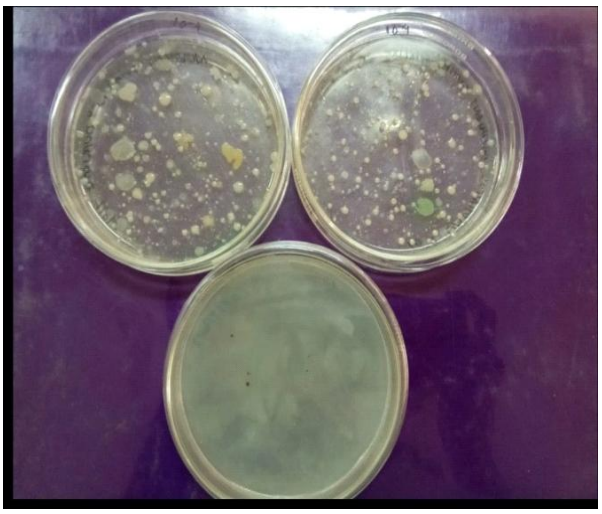
Isolation of pigment producing bacteria

The colonies in the dilutions 10^{-4},10^{-5},10^{-6} and 10^{-7} has pigmented colonies green, orange, yellow, which is denoted as Gb1,Gb2,Gb3.After streaking Gb1 gave green colour on citrimide agar plate, Gb2 and Gb3 gave orange and yellow colour on Nutrient agar plates(Table:1, Figure:3).

Table 1: Identification of pigmented bacteria

Dilution	Original /CFU	Duplicate /CFU
10^{-4}	424	421
10^{-5}	280	268
10^{-6}	112	108
10^{-7}	68	70

Figure 3: Isolation of pigment producing bacteria from garbage dumping ground soil sample for Dilution method



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The identification of pigment producing bacteria was done by Gram's nature, motility, morphology and biochemical characterization (Table: 2-6 and Figure: 4-12).

Table: 2 - Gram's staining of isolated bacteria

Isolates	Gram's staining
Gb1	Gram Negative rods
Gb2	Gram Negative rods
Gb3	Gram positive cocci

Key : Gb1- Garbage bacteria 1 , Garbage bacteria 2 , Garbage bacteria 3.

Table: 3 - Hanging drop technique performed in isolated bacteria

Isolates	Results
Gb1	Motile
Gb2	Motile
Gb3	Non Motile

Key : Gb1- Garbage bacteria 1 , Garbage bacteria 2 , Garbage bacteria 3.

Table: 4 - Biochemical characterization of bacteria Gb1

Biochemical Test	Result
Indole Test	Negative
Methyl Red Test	Negative
VogesProskauer Test	Negative
Citrate utilization Test	Positive
Catalase Test	Positive
Carbohydrate fermentation Test	Negative
Bacterial isolate	*Pseudomonas sp*

Table: 5 - Biochemical characterization of bacteria Gb2

Biochemical Test	Result
Indole Test	Negative
Methyl Red Test	Negative
Voges Proskauer Test	Positive
Citrate utilization Test	Positive
Catalase Test	Positive
Carbohydrate fermentation Test	Negative
Bacterial isolate	*Serratiaspp*

Table: 6 - Biochemical characterization of Gb3

Biochemical Test	Result
Indole Test	Negative
Methyl Red Test	Positive
Voges Proskauer Test	Positive
Citrate utilization Test	Positive
Catalase Test	Positive
Carbohydrate fermentation Test	positive
Bacterial isolate	*Staphylococcus spp*

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Figure 4: Quadrants streaking of green coloured pigmented bacteria.

Figure 5: Quadrants streaking of orange coloured pigmented bacteria.

Figure 6: Quadrants streaking of yellow coloured pigmented bacteria.

Figure 7: Identification of pigmented bacteria by Gram's Staining of Gb-1.

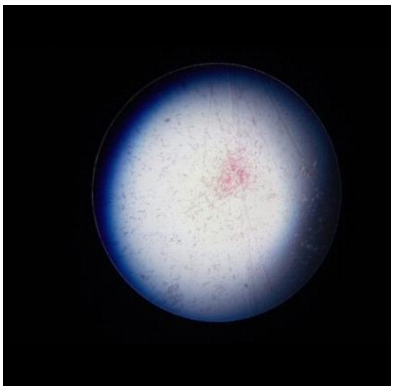

Figure 8: Identification of pigmented bacteria by Gram's Staining of Gb-2.

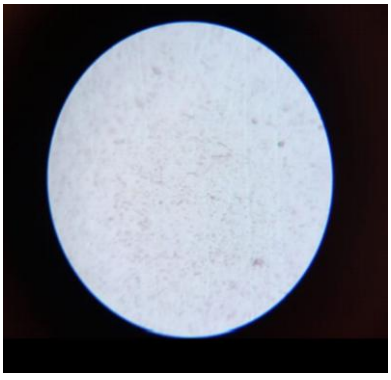

Figure 9: Identification of pigmented bacteria by Gram's Staining of Gb -3.

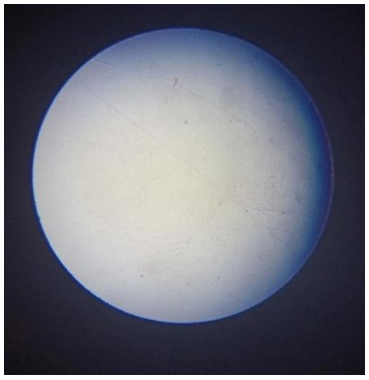

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Figure 10: Biochemical identification of Gb-1.

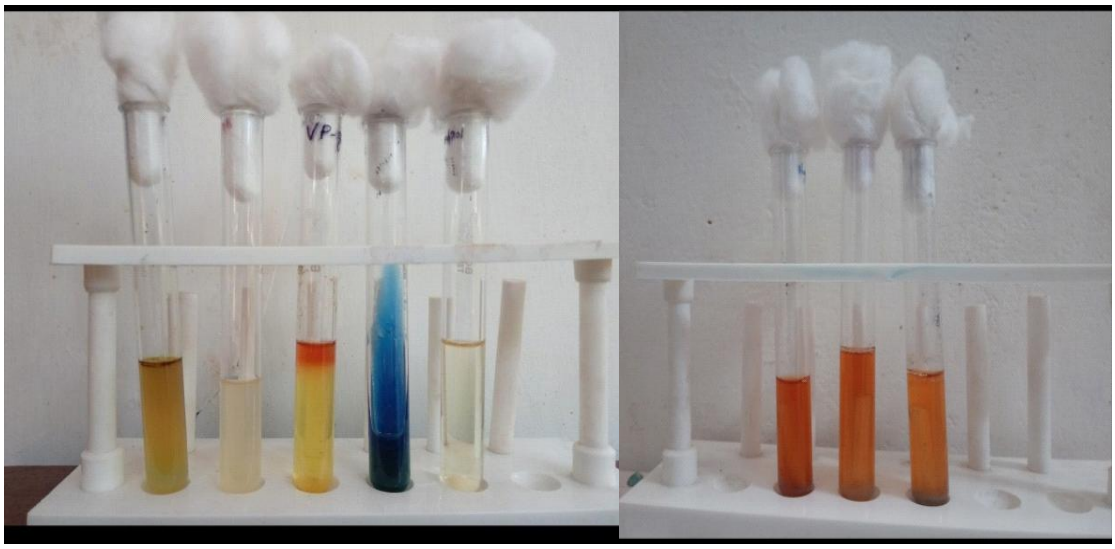
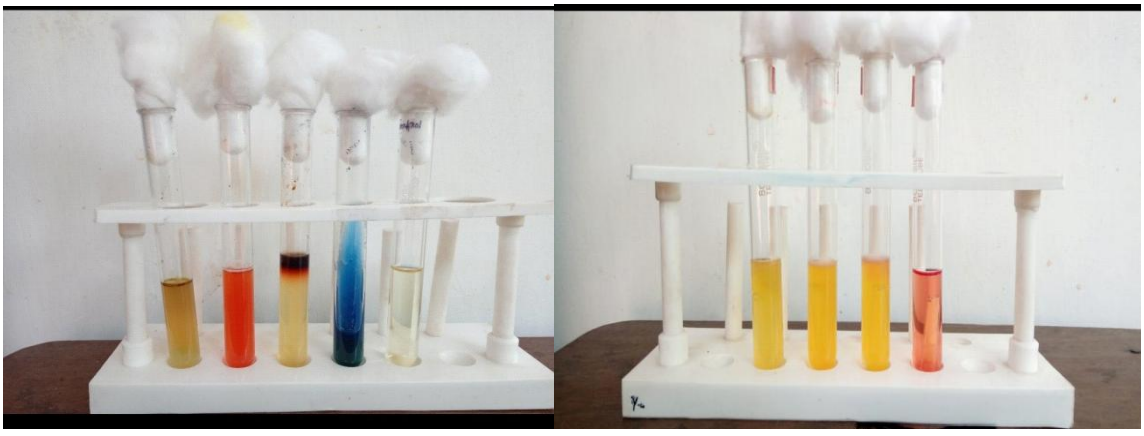


Figure 11: Biochemical identification of Gb-2.

Figure 12: Biochemical identification of Gb-3.



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Screening of pigment producing bacteria

After incubation the colour of broth changed in to Green, Orange (Figure: 13-15). Goswami *et al*. (2014) used liquid-liquid extraction method. *Serratia* is an intracellular pigment producing organism, so that the pigment was extracted by the lysis of cells.[12] These extracted pigment was applied on to the textile industry .The antimicrobial activity was showed against *E.coli*, 1mm zone of inhibition was showed. This indicates that the inhibitory metabolites produced by isolated organisms were intracellular.

Figure 13: Screening of pigmented bacteria for Green coloured pigmented broth of Gb-1

Figure 14: Screening of pigmented bacteria for Orange coloured pigmented broth of Gb-2.

Figure 15: Screening of pigmented bacteria for Yellow coloured pigmented broth of Gb-3.

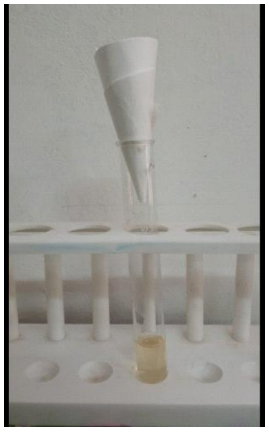
Extraction of pigment producing bacteria

The pigment of isolated pigment producing bacteria Gb1 an Gb3 were extracted by centrifuging at 6000 rpm for 15 mins and filtered extract with whatman no:1 filter paper and by the addition of organic solvents..The isolated pigment producing bacteria Gb2 was extracted by lysing the cells using centrifugation(Figure: 16-18) .

Figure 16: Extraction of Pigment for Green coloured extracted pigment of Gb-1

Figure 17: Extraction of Pigment for Orange coloured extracted pigment of Gb-2.

Figure 18: Extraction of Pigment for Yellow coloured extracted pigment of Gb-3.



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Sudhakar *et al*. (2015) used various solid and liquid media for production of pyocyanin pigment from *pseudomonas sp.*[13] Chloroform was added as organic solvents for pyocyanin extraction by centrifugation method and column chromatography used for purification. The antimicrobial activity tested against *E coli* , *S.aureus* and *klebsiella*.In this present study citrimide agar was added as organic solvents for pyocyanin from *Pseudomonas sp* by using centrifugation method, Methanol was used as the solvent. Pyocyanin is an extracellular pigment ,so it was Normally extracted then purified by whatman filter paper.

Antimicrobial activity

0.9mm zone of inhibition for Gb1 and 1mm zone of inhibition for Gb2 was observed on Nutrient agar plates against human pathogen *E coli* and is represented in the figure 9.Gb3 does not show antimicrobial activity (Figure: 19-21). The green coloured pigment was extracted. The extracted pigment shows antimicrobial activity against *E coli* 0.9mm zone of inhibition was showed. Then the *staphylococcus sp* was produced yellow colour pigment by centrifugation method, but this extracted pigment does not shows the antimicrobial activity against human pathogens.

Figure 19: Antimicrobial activity of extracted pigment of Gb-1

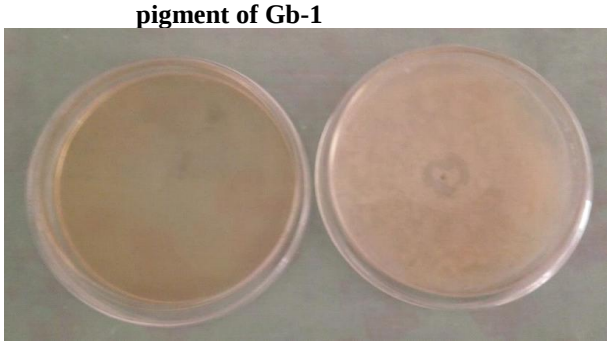
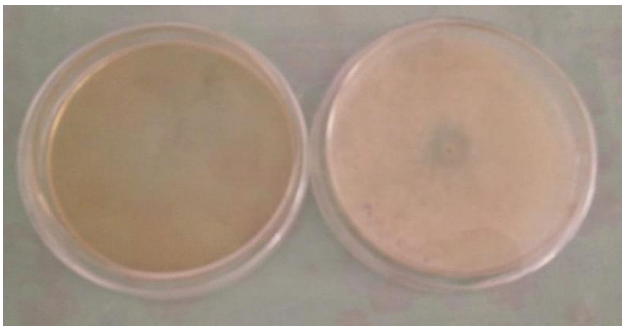


Figure 20: Antimicrobial activity of extracted pigment of Gb-2

Figure 19: Antimicrobial activity of extracted pigment of Gb-3



Application of bacterial pigment in textile industry

The extracted pigment of Gb2 is absorbed by the cotton cloth. The white colour of cloth is changed in to orange colour (Figure 22). The bacterias produce microbial pigments. The various coloured pigments are extracted by different ways like centrifugation, Filtration method. The extracted pigment can be applied into various industries mainly textile, food, pharmaceuticals.

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Figure 22: Application of extracted pigment of Gb 2 on to white coloured cotton cloth.

Conclusion

In this current study deals with an approach of developing new source of biocolours from easily cultivated bacterial species that can be further exploited at largescale industry.

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