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TEXTILE DYEING AND ANTIMICROBIAL ACTIVITY OF PIGMENTED MICROORGANISMS

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Introduction

The microorganisms are some of the most important living creatures. Because of their specific properties, there is a growing demand for more suitable medium to produce these natural pigments, especially for food coloring and nutritional supplementation (Downham and Collins, 2000).

Actinomycetes represent a ubiquitous group of microbes widely distributed in natural ecosystems around the world and especially significant for their role on the recycling of organic matter (Srinivasan *et al.*, 1991). Actinomycetes are one of the most important microorganisms that produce a wide variety of useful secondary metabolites, many of which are commercially important antibiotics and immunosuppressive compounds.

Rhodotorula species are pigmented basidiomycetous yeasts in the family Sporidiobolaceae easily identifiable by distinctive yellow, orange/red colonies (Prabhu et al., 2015; Kaur et al., 2009). Most *Rhodotorula* species produce colonies that are pink to coral in color but can also be orange to red on Sabouraud's agar.

Textile dyeing by natural pigments

Since millennia, humans have sourced pigments from plants, insects, animals and ores. It is suggested to exploit the potential of microorganisms such as fungi, bacteria and algae that are fast growing and have the potential of being standardized commercially (Sharma *et al.*, 2012). There are several microorganisms that can produce pigments, which are some of the important classes of secondary metabolites and are often referred to as biopigments. Microorganisms produce various pigments including carotenoids, melanins, flavins, quinones, prodigiosins and more specifically monascins, violacein or indigo (Khanafari *et al.* 2006; Venil and Lakshmanaperumalsamy 2009).

Actinomycetes are widely distributed in terrestrial environments and have long been a source of commercially useful enzymes and therapeutically useful bioactive molecules. Many of the presently used antibiotics such as streptomycin, gentamycin, rifamycin and erythromycin are the product of actinomycetes. Further they can produce an array of secondary metabolites, many of which have anti bacterial or anti fungal properties. In fact most antibiotics developed for human pharmaceutical use are actinomycete metabolites, many being derived from *Streptomyces* species (Goodfellow *et al.*, 1987). The *Streptomyces* are widely used in industry due to their ability to produce numerous chemical compounds including antibiotics, enzymes and anti tumor agents (Berdy, 1995).

Materials and methods

Isolation of pigmented actinomycetes and yeasts

Isolation and enumeration of actinomycetes and *Rhodotorula* present in the soil sample was performed by serial dilution plating technique using starch-casein nitrate agar and Sabourauds Dextrose Agar. The pigmented actinomycete was named as organism A and pigmented yeast cell was named as organism B. The organisms were identified using grams staining and various biochemical tests.

Production and extraction of the pigment

The culture of the organism A was inoculated onto SCN plates. It was allowed for incubation at 30°C for 7 days. This mycelium was scrapped from the agar surface and was extracted with methanol (Kojiri *et al.*, 1993) and the extract was concentrated by evaporating the solvent at room temperature. The evaporated extract was transferred to sterile eppendorf tubes and stored for further studies.

The 10 day old yeast cells, organism B grown on Sabouraud's agar plate were scraped with nichrome loop and were hydrolyzed with 1 N HCl in a water bath at 70° C for 90 minutes. The acid free cells were soaked overnight in methanol at room temperature. The extracted pigments were evaporated and stored in sterile eppendorf tubes (Pfander, 1992).

Preparation of textile material (Sagarika Devia & Perumal Karuppan, 2015)

The cotton fabric was scoured to remove any kind of impurities like fats, oil and any other finishing treatment given to materials so that they do not react during dyeing process. The scouring was carried out at boil for 60 min with 2 gpl (gram per litre) detergent maintaining the material liquor ratio 1:50. The scoured fabric was thoroughly rinsed with water to ensure complete removal of soap and subsequently dried at room temperature.

Dyeing of cotton and polyester fabric

The condensed pigment (20 mL) was redissolved in 100 ml distilled water. Pretreated cotton was dyed at material liquor ratio (MLR) of 1:50 at 70°C for 45 min. After dyeing, the fabric was treated with 1% acetic acid and washed thrice in running water. The dyed fabric was then rinsed with cold water and shade dried.

Percentage absorption of the dye

Percentage absorption of the fungal pigment by dyed fabrics was calculated using UV-Visible spectrophotometer (VARIAN, US) as per the following equation:

$$\text{Absorption (\%)} = \frac{\text{OD before dyeing- OD after dyeing x100}}{\text{OD before dyeing}}$$

Antimicrobial activity of the pigment

Antimicrobial activity of pigment was tested using the disc diffusion method using sterile Mueller – Hinton agar. The broth cultures of *Escherichia coli, Bacillus sp., Pseudomonas sp.,* and *Klebsiella sp.,* obtained from the Department of Microbiology,

Pazhassiraja College were taken and lawn cultured on the plate using sterile cotton swabs. Sterile discs were taken and the purified extract of the pigment to be tested were prepared at concentration of 100, 200, 300, 400 and 500 µg/ml in 10% DMSO and were added to wells cut on MHA. After incubation, the inhibition zones were measured in millimetres (Gerber and Lechevalier, 1976; Kojiri *et al.*, 1993).

Results and discussion

Isolation of pigmented actinomycetes and yeast cells

From the serially diluted soil sample typical pigmented actinomycete colonies producing bright red color were picked up and were further sub cultured on starch casein nitrate agar plates to obtain pure culture of the isolated sample (Hongjuan Zhao *et al.*, 2005). The yeast cells were orange in colour with a mucilaginous appearance on SDA plates. These samples were then characterized morphologically, culturally and biochemically.

Morphological identification by Gram staining

The result of gram staining of isolated culture, organism A showed gram positive filamentous rods and organism B showed gram positive budding oval cells.

Cultural characterization

The growth of the organism on starch casein nitrate agar showed the characteristic powdery or chalk- like appearance of Actinomycetes on agar surface. The bright red pigmentation was found on the reverse side of plate and was intracellular in nature. The mature spores were grey in color (Plate 1 and 2). The colony characteristics of the isolated yeast cells showed mucoid orangish pigmented colonies with a sour odour. They appeared as large cells with ellipsoidal form and peripheral budding (Plate 3).

The synthesis of different natural carotenoids by several yeast species such as the basidiomycetous genus Rhodotorula has led to consider these microorganisms as potential pigment sources (Frengova *et al.* 1994). Carotenoid production by yeast can become industrially feasible using low-cost agro-industrial byproducts as carbon sources (Buzzini *et al.*, 2001) and minimizing environmental problems related to residuesand effluent disposal (Loehr 1974).

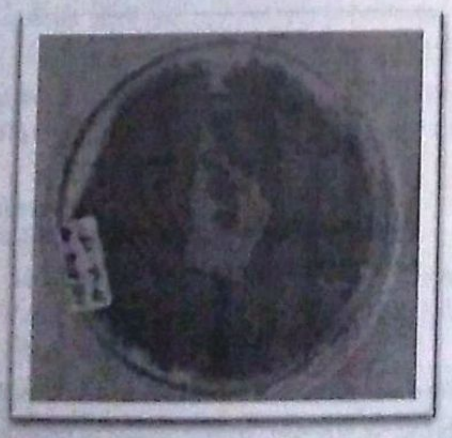


Plate 1: Organism A

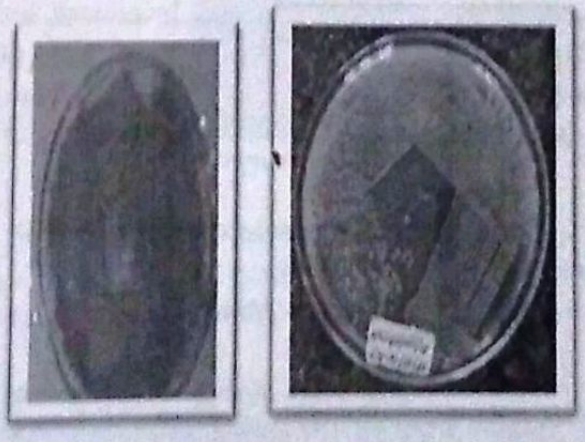


Plate 3: Organism B

Biochemical characterization of the isolate

The results of biochemical characterization of the isolates are given in Table 1.

Sl. No	Morphological and biochemical characters	Organism A	Organism B
1	Gram staining	Gram positive	Gram positive
2	Pigment production	+	+
3	Indole	-	-
4	Methyl red	-	-
5	Voges-Proskauer	-	-
6	Citrate utilization	+	-
7	Oxidase	-	-
8	Catalase	+	-
9	Urease	-	+
10	Gelatinase	-	+
11	Glucose	+	+
12	Lactose	-	-
13	Sucrose	-	+
14	Fructose	-	-

[+] Positive [-] Negative

Table: 1

Morphological and biochemical characterization of the isolate

Pigment production and extraction

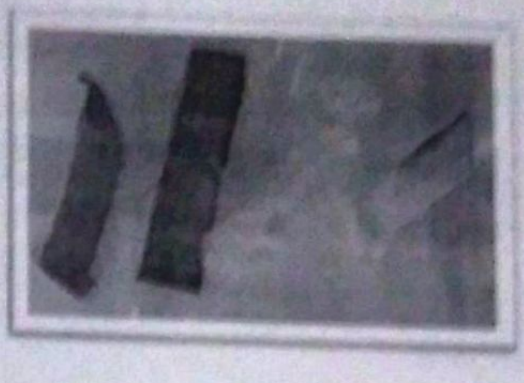
The pigment produced by organism A grown on starch casein nitrate broth was extracted by adding methanol (Plate 4) and organism B by the method of Pfander, 1992 (Plate 5).

Plate 4: Pigment extract of A in methanol

Plate 5: Pigment extract of B in methanol

Textile colouring and Absorbance

The maximum dyeing of the pigments from organism A and B was observed on cotton fabrics (Plate 6). The optical density and absorption at 520 nm of the same is tabulated (Table 2).



Microbes can be exploited as one of the natural sources of textile dyes. These multiply very fast and are capable of growing on large scale on a variety of raw materials requiring limited space.

Plate 6: Textile dyeing by pigments

Fabric	Optical density				Absorption %	
	Before dyeing		After dyeing			
	Organism A	Organism B	Organism A	Organism B	Org A	Org B
		Pigment A		Pigment B		
Cotton	0.52	0.56	0.38	0.43	26.92	23.21
Polyester	0.52	0.56	0.41	0.46	21.15	17.85

Table 2: Textile dyeing property of pigments from organism A and B

Antimicrobial activity of the pigment

The pigment of the isolated actinomycetes and the yeast cells were dissolved in 10% dimethyl sulfoxide (DMSO). . On antimicrobial studies, the activity of the pigment was shown against both gram positive and negative organisms. Organism A produced good zones of inhibition against *E. coli, Klebsiella sp.,* and *Bacillus* sp., with inhibition zones of 10 mm, 0.5 mm, 10 mm at highest concentration of 500 µg/ml respectively and organism B showed activity against *Bacillus* and *E. coli* with inhibition zones of 1.5mm and 0.5mm at highest concentration of 500 µg/ml respectively. No activity of the pigments was observed with *Pseudomonas sp.,*

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