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Selection of Pigment (Melanin) production in Streptomyces and their application in Printing and Dyeing of Wool Fabrics

Ali M. Amal[1], Keera A. Abeer[1], Helmy M. Samia[1], Abd El-Nasser H. Nadia[1], Ahmed K.A.[2] and El-Hennawi H.M.[2]

[1]Microbial Chemistry Dept., Genetic Engineering and Biotechnology Division, National Research Center, Dokki, Cairo, EGYPT
[2]Chemistry of Dyeing, Printing and Auxiliaries Department, Textile Division, National Research Centre, Cairo, EGYPT

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Abstract

Four strains among 30 Streptomyces were isolated producing a diffusible different pigment in eight different media. Streptomyces virginiae was the most producer of a diffusible dark brown pigment on both peptone-yeast extract and tyrosine liquid medium. Some factors affecting on the pigment production were studied. The optimum pH was observed at 6 with 10 days of the Streptomyces age and five days of incubation on a rotary shaker (180 rpm) at 30 ∘C. The intensity of the pigment was affected by addition of carbon, nitrogen and phosphorus sources. Co, Mg and pb SO_4 were increased the intensity of the pigment production. The pigment produced at optimum condition had been used to dye and print the wool fabrics, the color strength values and fastness properties of samples were investigated. At the optimum conditions the dyed and printed wool fabrics posses color strength values 12, 10.5 respectively and very good fastness properties of washing,, perspiration and light fastness. The antimicrobial activity of the extracted pigment has been investigated.

Keywords: Streptomyces, pigment, melanin, optimum, production, application on wool fabrics, antimicrobial potentialities.

Introduction

Actinomycetes are characterized by the production of various pigments on natural or synthetic media. These pigments are usually described in terms of various shades of blue, violet, red, rose, yellow, green, brown and black. The pigments maybe dissolved in to the medium or it may be retained in the mycelium. Actinomycetes had known to be produced various kinds of antibiotics and moreover these antibiotics include many pigments[1]. Production of pigments by actinomycetes has been utilized as an important cultural characteristic in describing the organisms. Nevertheless, very little is known about the exact chemical nature of pigments, because the formation of pigment is influenced by the pH of the medium, aeration, temperature of the growth and carbon and nitrogen sources. Actinomycetes also synthesized and excrete dark pigments melanin or melanoid, which considered to be useful criterion for taxonomical studies [2-4]. These pigments are not sensitive to change in pH[5]. The actinomycetes, designated strain GIMV4.0001 produced a white aerial mycelium and violet-blue diffusible pigment on Gause's synthetic agar[6]. Another actinomycete designated as strain GIMN4.002T, produced white aerial mycelium and dark-blue diffusible pigment on Gause's synthetic agar[7]. The textile industry produces and uses approximately 1.3 million tonnes of dyes, pigments and dye precursors, valued at around $23 billion, almost all of which is manufactured synthetically. However, synthetic dyes have some limitations, primarily, (i) their production process requires hazardous chemicals, creating worker safety concerns, (ii) they may generate hazardous wastes, and (iii) these dyes are not environment friendly [8].

Considering the toxic effects of the synthetic dyes there has been a renewed effort to study and implement the various natural dyes in the dyestuff industry. Primarily there are three categories of natural dyes. Firstly those are derived from plants like indigo. Second, the ones that are obtained from animal sources called Cochineal, and those that are got from minerals (Ocher). Natural dyes can provide the much needed alternative to the complex world of chemical dyes [9-10]. The global interest and demand in application of the fungal pigments such as carotenoids, flavonoids, betalains, quinones and some tetrapyrroles in dyeing of cotton, silk and wool has been reported in several studies and increased due to the toxicity problems caused by those of the synthetic origin[11]. The production and evaluation of microbial pigments as textile colorants is currently being investigated[11]. Fungi are more ecological interesting source of pigments, since some fungal species are rich in stable colorants such as anthraquinone[12-13].This research explores methods where natural dyes are produced from fungal species

The pigment producedfrom *Sclerotinia* sp.was applied on cotton shades, which were confirmed using the standard color code index[14]. Melanins are frequently used in medicine, pharmacology and cosmetics preparations[4]. However, no reports concerning the production of brown pigment produced by *Streptomyces* strains is available.

Aim of Work: The purpose of this study is to select the *Streptomyces* strain which is capable of producing brown pigment. This work deals with the fermentation, selection of the brown pigment and cultural condition, nutritional requirements for brown pigment production by *Streptomyces*. The efficiency of brown pigment produced from *Streptomyces* strain in dyeing and printing of wool fabrics and fastness properties have been investigated. The antibacterial activity of the extracted pigment against bacteria, yeast and fungi.has been studied

Material and Methods

Organisms and culture conditions: A total thirty streptomycetes isolated from soil samples and identified by Keera were revived by inoculating 1 ml of heavy spore suspension (7 days old slants) into a 250 ml conical flask containing 50 ml of culture medium (g %: 2, starch; 0.2, KNO_3; 0.1, K_2HPO_4; 0.05, NaCl and 0.05, $MgSO4.7H_2O$, pH 7.0 adjusted before autoclaving) followed by incubating at 28ºC in a rotary shaker (180 rpm) for 5 days at pH 7.0. The cultures were screened for ability to grow and produce pigment in a defined eight different media such as starch nitrate, glycerol asparagine, dox, fish meal extract, oat meal extract, malt extract, tyrosine and peptone-yeast extract-iron media as mentioned in International *Streptomyces* Project (ISP)[15].

Streptomyces strain of high pigment producer was identified as *S. virginiae*[16] which had hooked spore chain, smooth spore surface, dark red grey aerial mycelium, red grey substrate mycelium melanin +ve and anti-fungal antibiotic (table 1).

Table-1
Antimicrobial Potentialities of the broth produced by S. virginiae

Test Organisms	Zone of inhibition (mm)
Aspergillus niger	21
Aspergillus terreus	15
Macrophomina phaseoli	15
Helminthosporium turcicus	26
Fusarium oxysporium	24
Diplodia oryzae	16
Rodotorula minuta	17

Aspergillus flavus, Botrytis allii, Trichoderma viride, Sacchromyces carlsbergensis, Sacchromyces cerevisiae, Candida albicans, Candida Tropicalis and Candida pseudotropicalis gave negative results.

Some optimum conditions for pigment produced by the best *Streptomyces* strain were investigated. The effect of age of microorganism, incubation periods, aeration and initial pH, different concentration of peptone, different carbon sources and heavy metals on the pigment produced by the best strain of *Streptomyces* were determined.

Melanin estimation: Melanin pigment was estimated from peptone-yeast extract iron medium according to Dastager *et al*.

U V/Visible spectrophotometry: The absorption spectra of the dyes were recorded using Perkin Elmer Double Beam Spectrophtometer. The maximum absorption peaks of each dye were then detected in the visible region for determination of lambda max. and for further comparison with other dyes.

Dyeing experiment: Lab scale dyeing experiments were carried out on wool fabrics using the produced dyes without mordanting. The dye bath was prepared with the produced dye at a liquor ratio 50: 1 and with 1 g/l amphoteric leveling agent (Albegal B), pH was adjusted to pH 1.0. Dyeing was started at 50°C for ten min., then the dye bath temperature was raised to boil over 30 min and the dyeing continued for 45 min. After dyeing the temperature was lowered to 60 °C, then the dyed samples were rinsed and washed off in an aqueous solution 2 g/l non-ionic detergent (Hospital CV) at 60°C using a liquor ratio 50: 1 for 30 mm, rinsed and dried.

Printing experiment: Lab scale printing experiments were carried out on wool fabrics using the produced dyes without mordanting. The printing paste 100 gm. consists of 3gm. of sodium alginate as thickener, 3 ml of produced dye and 96 ml of water

Color strength: The reflectance values of the dyed fabrics were measured using a Data Color SF 600+. Relative color strengths (K/S values) were determined using the Kubelka-Munk equation[17].

$$K/S = (1-R)^2/2R$$

Where R is the decimal fraction of the reflectance of dyed fabric, K is the absorption coefficient, and S is the scattering coefficient.

Fastness testing: The dyed samples were tested according to ISO standard methods[8]. The specific tests were as follows: ISO 105-C02, color fastness to washing; ISO 105-E04, color fastness to perspiration, and ISO 105-B02, color fastness to light (carbon arc)[18].

Antimicrobial Potentiality: The antimicrobial activities were measured as described by[19]. The pigment was precipitate by ammonium sulphate at different concentrations 20%, 25%, 30%, 35% and 65%. The antimicrobial activities of all precipitated were determined against bacteria (gram –ve and +ve), yeast and fungi.

Results and Discussion

A comparative study was performed between some available cultures of grey series of *Streptomyces* to assess their ability to produce melanin pigment on eight different media such as starch nitrate, glycerol asparagines, dox, fish meal extract, oat meal extract, malt extract, tyrosine and peptone-yeast extract-iron liquid media. The results are summarized on table 2. The studied *Streptomyces* strains could not produce any pigment on starch nitrate, glycerol asparagines, dox and oat meal extract media. The highest level of the pigment formation was detected in culture of *Streptomyces virginiae* with peptone-yeast extract-iron followed by tyrosine liquid medium. Therefore, *S. virginiae* was selected for further studies using peptone-yeast-extract-iron liquid medium.

The optimum conditions Effect of *Streptomyces virginiae* age on pigment formation and color strength value (K/S): The age of *Streptomyces virginiae* (1-10 day's) was detected for pigment formation on peptone-yeast-extract-iron liquid medium. We observed that the fourth day of *S. virginiae* age was the best day for pigment formation (figure 1).

The color strength value (K/S) of wool dyed sample increased by increasing the age of *S. virginiae* till it reached to value (5.20) at age 4th day, then slightly decrease during the period from 5th to 10th day figure 2, the result represents a good relationship between pigment production expressed as O.D. (Optical Density) and color strength of dyed wool sample. Table 3 illustrates the fastness properties of dyed wool fabrics to washing, perspiration (acidic and alkaline) and light. The results indicate that all the dyed samples showed good to very good ratings. The 2nd day exhibited the best results of fastness properties

Table-2
Screening for melanin pigment produced by different strains of streptomycetes on eight different liquid media incubated on a rotary shaker (180 rpm) for five days at 30 °C and pH 7.0

No. of *Streptomyces*	Liquid medium	O.D at 480 nm
s. virginiae	Fish meal extract	0.000
	Soy-bean meal extract	0.000
	Tyrosine	1.500
	Peptone-yeast-extract-iron	2.248
S. X2	Fish meal extract	0.198
	Soy-bean meal extract	1.113
	Tyrosine	0.150
	Peptone-yeast-extract-iron	0.377
S. 19	Fish meal extract	0.243
	Soy-bean meal extract	0.000
	Tyrosine	1.492
	Peptone-yeast-extract-iron	1.863
S. N7	Fish meal extract	2.064
	Soy-bean meal extract	0.000
	Tyrosine	1.500
	Peptone-yeast-extract-iron	0.272

Starch nitrate, glycerol asparagines, dox, oat meal extract liquid media gave negative results.

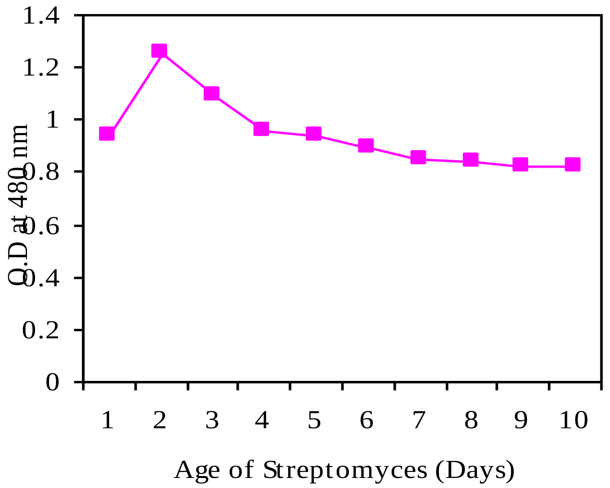

Figure-1
Effect of *S.virginiae* age on pigment formation

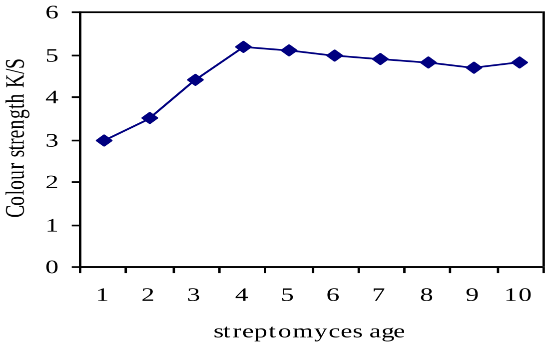

Figure-2
Effect of *S.virginiae* age on the color strength

Effect of aeration on pigment formation by *Streptomyces virginiae* and color strength (k/s): It is clear from results represented in figure 3 that, maximum O.D of the pigment formation was observed in the volume 50 ml fermented medium of *S. virginia* / 250ml conical flask, then it decreases by increasing the medium volume. Figure 4 represents that the highest color strength value is detected at 50 ml of *S. virginiae* fermented area/250 ml then it decreases with increasing the medium volume.

Results presented in table 4, illustrate fastness properties of the dyed wool samples (washing, perspiration and light). The samples showed good to very good fastness properties. Wool samples dyed with dye sample obtained at volume 50 ml fermented medium of *S. virginiae* / 250ml conical flask, exhibited the best fastness properties. These features indicate that there was a good relation between K/S value and fastness properties of dyed wool samples. The results represent that there was a relation between K/S value and pigment production presented as optical density.

Effect of initial pH on pigment formation by *Streptomyces virginiae:* It is evident from results represented in figure 5 that, the optimum pH of pigment formation was detected at pH 6 by *S. virginiae*. Low pigment formation was observed with acidic pH while, alkaline pH gave moderate pigment formation. Table 5 represents the fastness properties and color strength of dyed wool sample at different pH, it is regarded that the dyed samples showed good to very good rating of fastness properties, acidic medium shows low color strength, the highest value of the color strength (k/s) is shown at pH 6 and 7, at higher pH slightly decreasing of K/S is observed (Figure 6).

Effect of incubation period and color strength on pigment production by *Streptomyces virginiae:* The time course of pigment formation by *Streptomyces virginiae* was studied in twelve days. The maximum level of pigment formation was observed at 10th day of incubation period and then slowly declined (Figure 7). It is noticed from figure 8 that high color strength value (K/S) of dyed wool fabrics is observed at the 10th day of incubation period, then no remarkable increase in K/S value with 11th and 12th days.

Table 6 represents that, the results of all the dyed samples showed good to very good ratings, the period from 6th to 10th days exhibited the best results of fastness properties. This observation may be attributed to increasing the dye fiber affinity as well as the dye-fiber stability relative to the produced dye at the 10th day of incubation period. As a fiber, wool is heterogeneous, both chemically and physically. In case of wool without the damage in cuticle cells, it is assumed that the dye uptake in the early stage of adsorption is subject to the dyeing behavior of intracellular regions of cuticle-cuticle (surface) layer[17]. Dye most readily enters the fibers by diffusion through the intracellular region between the scale cells of the wool fibers, penetrates fairly rapidly into the non keratinous endocuticle region of the surface layer, which soon reaches equilibrium with the dye in the outer solution. Within the cuticle cells, the endocuticle and then the exocuticle becomes colored, as the dye migrates through the intracellular cement and penetrates into the cells from their undersides[20].

Effect of carbon sources supplemented with the utilized medium on pigment formation by *Streptomyces virginiae* and color strength: Supplementation of the peptone-yeast-extract-iron medium with different carbon sources on pigment formation by *S. virginiae* was studied. The results are summarized in table 7. The highest level of pigment formation By *S. virginiae* was detected with L-arabinose followed by glycerol, fructose, mannose, manitol and glucose. The lowest level of the pigment formation was observed with galactose. The effect of carbon sources supplemented with the utilized medium has no remarkable change on the color strength of dyed wool sample all the color strength values lay between 11. - 11.8

Table-3
Effect of pigment formation by *Streptomyces virginiae* age on color strength and fastness properties of dyed wool fabrics using produced pigment

S. virginiae age			1	2	3	4	5	6	7	8	9	10
Color			Yellow brown	Yellow brown	Brown	R. brown	R. brown	R. brown	brown	Brown	R. brown	R. brown
λ Max. (nm)			472	473	474	477	476	475	474	475	467	468
K/S Values			3	5.2	4.4	5.0	4.2	4.7	4.9	4.8	4.8	4.9
Wash fastness		*Alt	3	3-4	3-4	4	4	4	4	4	3-4	3-4
		*SC	3-4	3-4	4	4	4	4	4	4	3-4	3-4
		*SW	3	3	3-4	4-5	4-5	4	4	4	3-4	3-4
Perspiration fastness	Acid	Alt	3	3	3	4-5	4	4	4	4	4	4
		SC	3	3	3	4-5	4	3-4	4	4	4	4
		SW	3	3-4	3-4	4-5	4-5	4	3-4	4	4	4
	Alkali	Alt	3	3	4	5	4-5	4-5	4	4	4	4
		SC	3-4	3-4	4	4	4	3-4	4	4	4	4
		SW	3	3-4	4	4	4	3-4	4	4	4	4
Light fastness			3-4	4	4	5	4-5	4	4-5	4	3-4	3-4

*Alt.=Alternation, *SC= Staining on cotton, *SW=Staining on wool

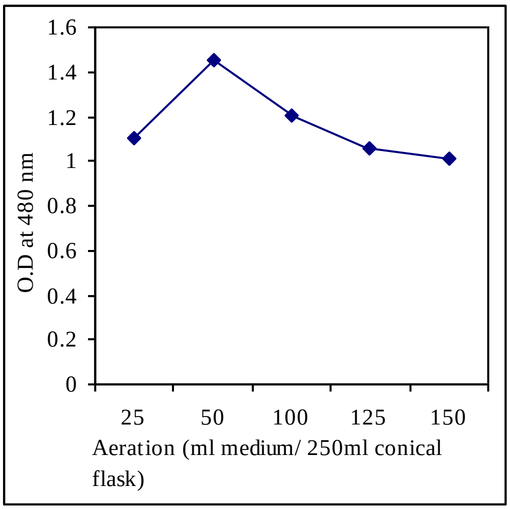

Figure-3
Effect of aeration on pigment formation by *S. virginiae*

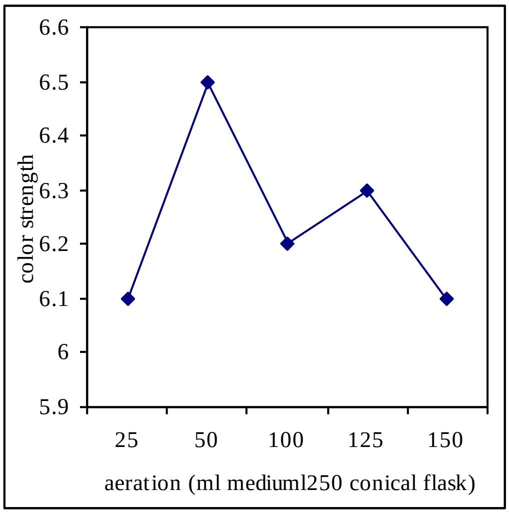

Figure-4
Effect of *S. virginiae* aeration on the color strength K/S

Table-4
Effect of aeration on pigment formation by *Streptomyces virginiae* on the color strength K/S and fastness properties of dyed wool fabrics using produced pigment

Streptomyces virginiae aeration volume			25	50	100	125	150
λ Max. (nm)			470	477	475	473	472
K/S Values			6.1	6.5	6.2	6.3	6.1
Wash fastness		Alt	3	4	3-4	4	4
		SC	3-4	4	3-4	4	4
		SW	3-4	4	4	4	4
Perspiration Fastness	Acid	Alt	4	4-5	4	3-4	4
		SC	4	4-5	4-5	4	4
		SW	4	4-5	4-5	5	5
	Alkali	Alt	4-5	4	4	4	4
		SC	4	4	4-5	4-5	4
		SW	4	4-5	4	4-5	4
Light fastness			4	5	4-5	5	5

Table-5
Effect of initial pH on color strength and fastness properties of dyed wool fabrics using produced pigment by *Streptomyces virginia*

initial pH			1	2	3	4	5	6	7	8	9	10
Color			Light brown	Light brown	yellow Brown	Brown	Brown	Dark brown	Dark brown	R. brown	R. brown	R. brown
λMax. (nm)			472	473	475	474	477	480	479	479	475	477
K/S Values			6.5	7.1	7.3	7.68	8.9	11.5	10.9	9.6	9.8	10.1
Wash fastness		Alt	3-4	3-4	4	4	3-4	4-5	4	4-5	4-5	5
		SC	3	3-4	3-4	4	4	4	4	4	4	4-5
		SW	3-4	3-4	3-4	4	4	4	4	4	4-5	4-5
Perspiration Fastness	Acid	Alt	4	4	4	4	4	4-5	4	3-4	5	4
		SC	4	4	4	4-5	4-5	4	3-4	4	4-5	4-5
		SW	4	4	4	4-5	5	4-5	4	4-5	5	4
	Alkali	Alt	4	4	4	4	5	4	4-5	4-4	4	4
		SC	4	4	4	4	4	4-5	4	4	4-5	4
		SW	4	4	4	4	4	4	4	4		4-5
Light fastness			3-4	4	4	4	4-5	4-5	5	4-5	4	5

Effect of different concentration of peptone on pigment formation and color strength by *Streptomyces virginiae:* Different concentrations of peptone were studied on the pigment formation by *Streptomyces virginiae*. The results obtained in figure 9 show that, the maximum level of the pigment formation was observed with 1.5 g% peptone. Peptone concentration has no any effect on the color strength of dyed wool sample. table 8 represents the fastness properties and color strength value of dyed wool fabrics at different concentration of peptone the result showed that all samples have good fastness properties the highest value of color strength are exhibited at 1.5 and 1.75 of peptone concentration.

Effect of heavy metal ions on pigment formation by *Streptomyces virginiae*: Some divalent metals salts were added to the utilized medium after sterilization each in concentration of 0.1 mM. As shown in figure 10, Co^{++}, Hg^{++} and Pb^{++} gave high concentration of the pigment formation by S. *virginiae*. Moderate concentration of the pigment formation was observed with Ni^{++} and Cu^{++}. Mn^{++} gave low concentration of the pigment formation. Changing the heavy metal does not make significant effect on the color strength of dyed wool sample.

Fastness properties of printed wool fabric at optimum conditions: Table 9 illustrates the fastness properties of printed wool fabric using dye produced by *Streptomyces virginiae* at the optimum conditions. The result indicated that, a good to very good fastness properties rating and the color strength value is 10.5.

Antimicrobial potentialities: The dark brown pigment produced by *S. virginiae* was precipitated by different concentration of ammonium sulphate from the fermented broth of peptone-yeast-extract-iron liquid medium produced by *Streptomyces virginiae*. Antimicrobial activities of the dark brown pigment are illustrated in Table 10. It showed activity with fermented broth against Gram –v and +v bacteria (*Pseudomonase aeurogenosa* and *Staphylococcus aureuse*) as well as fungi (*Fusarium oxysporium, Botrytis allii*, *Diplodia oryzae*, *Aspergillus flavus*, and *Aspergillus niger*). Pigment precipitated at 20% - 35% concentration of ammonium sulphate gave antimicrobial activity with *Aspergillus niger*. Antimicrobial activity was observed at 20% ammonium sulphate with *Botrytis allii*, *Diplodia oryzae*, *Aspergillus flavus*. Some differences in our results observed with the data obtained with Keera, 2004 (Table 1). The obtained results suggest that antimicrobial substance is broad spectrum.

The protective role of melanin against UV Radiation (UVR): Human skin is repeatedly exposed to UVR that influences the function and survival of many cell types and is regarded as the main causative factor in the induction of skin cancer. in some parts of the polymer molecule and a gradual loss UV radiation is one of the major causes of degradation of textile materials, which is due to excitations in some parts of the polymer molecule and a gradual loss of integrity, and depends on the nature of the fibers. It has been traditionally believed that skin pigmentation is the most important photo protective factor, as melanin, functioning as a broadband UV absorbent, so it is expected that, the extracted melanin pigment exhibits from good to excellent ultra violet protection factor (UV P)[21-22].

Table-6
Effect of incubation period on color strength and fastness properties of dyed wool fabrics using produced pigment by *Streptomyces Virginia*

initial pH			1	2	3	4	5	6	7	8	9	10
Color			Light brown	Light brown	yellow Brown	Brown	Brown	Dark brown	Dark brown	R. brown	R. brown	R. brown
λMax. (nm)			472	473	475	474	477	480	479	479	475	477
K/S Values			6.5	7.1	7.3	7.68	8.9	11.5	10.9	9.6	9.8	10.1
Wash fastness		Alt	3-4	3-4	4	4	3-4	4-5	4	4-5	4-5	5
		SC	3	3-4	3-4	4	4	4	4	4	4	4-5
		SW	3-4	3-4	3-4	4	4	4	4	4	4-5	4-5
Perspiration Fastness	Acid	Alt	4	4	4	4	4	4-5	4	3-4	5	4
		SC	4	4	4	4-5	4-5	4	3-4	4	4-5	4-5
		SW	4	4	4	4-5	5	4-5	4	4-5	5	4
	Alkali	Alt	4	4	4	4	5	4	4-5	4-4	4	4
		SC	4	4	4	4	4	4-5	4	4	4-5	4
		SW	4	4	4	4	4	4	4	4		4-5

Table-7
Effect of carbon sources supplemented with peptone-yeast-extract-iron medium pigment formation by *Streptomyces virginiae*

Different carbon Sources	O.D at 480 nm	Color strength Value
Glucose	2.165	11.3
Fructose	2.253	11.6
Cellobiose	1.994	11.4
Manitol	2.168	11.6
L-arabinose	2.379	11.8
Galactose	1.467	11.0
Mannose	2.183	11.4
Lactose	1.761	11.1
Sucrose	1.922	11.3
Glycerol	2.282	11.7
Starch	1.614	11.1

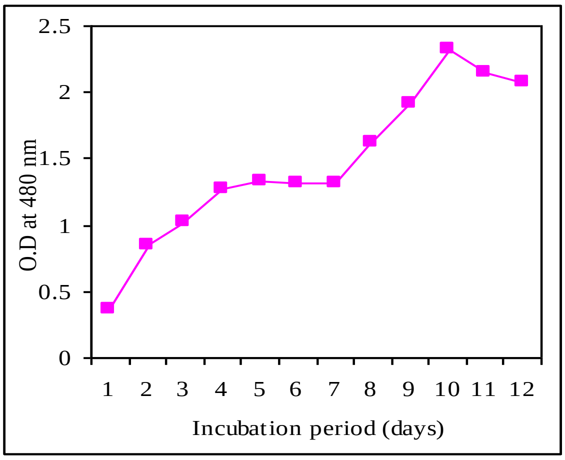

Figure-7
Effect of incubation period on pigment formation by *S. virginiae*

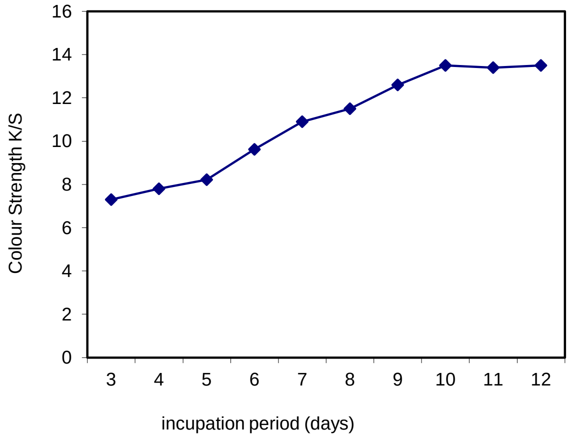

Figure-8
Effect of incubation period on color strength by *S. virginiae*

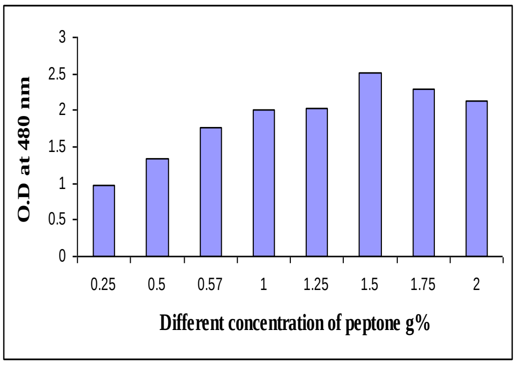

Figure-9
Effect of different concentration of peptone on pigment formation by *Streptomyces virginiae*

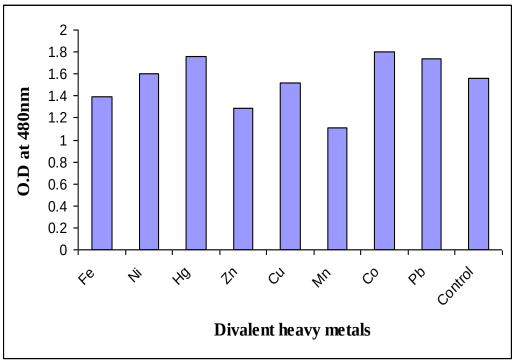

Figure-10
Effect of divalent heavy metals on pigment formation by *Streptomyces virginiae*

Table-8
Effect of different concentration of peptone on color strength and fastness properties of dyed wool fabrics using produced pigment by Streptomyces virginiae

Peptone concentration g/l			0.25	0.5	0.75	1	1.25	1.5	1.75	2.0
Color			Yellow Brown	Yellow brown	brown	Reddish Brown	Reddish brown	Reddish brown	Brown	brown
λMax. (nm)			452	473	474	477	476	475	474	475
K/S Values			4.1	5.0	6.1	9.2	9.5	12	11.3	11
Wash fastness		*Alt	3	3-4	3-4	4	4	4	4	4
		*SC	3-4	3-4	4	4	4	4	4	4
		*SW	3	3	3-4	4-5	4-5	4	4	4
Perspiration Fastness	Acid	Alt	3	3	3	4-5	4	4	4	4
		SC	3	3	3	4-5	4	4	4	4
		SW	3	3-4	3-4	4-5	4-5	4	5-4	4
	Alkali	Alt	3	3	4	5	4-5	4-5	4	4
		SC	3-4	3-4	4	4	4	4	4	4
		SW	3	3-4	4	4	4	4	4	4
Light fastness			3-4	4	4	5	4-5	5	4-5	4

Table-9
Fastness properties of printed wool fabric at optimum conditions

Color			Dark brown
λMax. (nm)			482
K/S Values			10.5
Wash fastness		*Alt	4-5
		*SC	4-5
		*SW	4-5
Perspiration Fastness	Acid	Alt	4-5
		SC	5
		SW	4-5
	Alkali	Alt	4
		SC	4
		SW	4-5
Light fastness			5-6

Table-10
Antimicrobial spectrum of *Streptomyces virginiae* pigment precipitated at different concentration of ammonium sulphate

Test organism	Zone of inhibition (mm)					
	Control broth	Concentration of ammonium sulphate				
		20%	25%	30%	35%	40%
Pseudomonase aeurogenosa	25	0	0	0	0	0
Staphylococcus aureuse	20	0	0	0	0	0
Fusarium oxysporium	25	0	0	0	0	0
Botrytis allii	12	17	0	0	0	0
Diplodia oryzae	14	20	0	0	0	0
Aspergillus flavus	14	28	0	0	0	0
Aspergillus niger	16	20	18	18	18	0

Bacillus cereus, Bacillus subtilis, Candida albicans and *Sacchromyces cerevisiae* gave negative results.

Conclusion

The above mentioned results indicate that the optimum conditions for production of dark brown dyes which give highest color strength value and very good fastness properties of dyed and printed wool fabrics at using peptone-yeast-extract-iron liquid medium are *Streptomyces virginiae*, age 2 days, initial pH 6, incubation period 10 days and peptone concentration 1.5. Pigment precipitated at 20% - 35% concentration of ammonium sulphate gave antimicrobial activity with *Aspergillus niger*. From the previous results, the dye produced from *S. virginiae* is very suitable for dyeing and printing wool fabrics as a replacer of synthetic dyes which has big environmental problems. In future the antimicrobial substance will be applied in the medical fabrics.

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