Enhancement of colour yield of pigment produced by Trichoderma pseudo kii nii pii, Alternaria alternata and Curvularia lunata

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Abstract:

Three fungal species *Trichoderma psuedo kii niipii, Curvularia lunata* and *Alternaria alternate* were isolated and produced yellow, olive green and melanin (peach) color pigment when grown in potato dextrose agar medium (PDA) at stationary condition. Optimum fermentation parameters for maximum pigment production are medium-rice water, pH 5.6, temperature 28ºC, time of incubation 10 days. Dextrose enhances pigment production whereas ammonium sulphate inhibits the process.

Keywords: *Pigment, Fungus, Dyeing, Natural dye*

1. Introduction

Colour is a vital constituent and is probably one of the first characteristics perceived by the senses. With the increasing awareness of toxicity of synthetic colours, demand for pigments from natural sources has increased (Babu and Shenolikar, 1995; Khannaanf Singh, 1975). Natural colours are generally extracted from fruits, vegetables, roots and microorganisms and are often called “biocolours” because of their biological origin (Pattnaik et al., 1997). There is increasing demand for natural colours in the food, pharmaceuticals, cosmetics, textile and in printing dye industry. Microbial pigments are a promising alternative to other colour additive extracted from

vegetables or animals because they are considered natural, pose no seasonal production problem and show high productivity. Pigment producing microorganisms are yeast, fungi, bacteria, micro algae and are quite common in Nature. Microorganisms produce various pigments like carotenoids, melanins, flavins, monascins (DufosseL., 2009)

In a study conducted by Chiba et al (2006), a magenta pigment closely related to PhobaHerbarum was grown by a fungal strain in the presence of nylon 6 fibres on Czapek's Agar medium. Nagi et al (2006) described two anthraquinone compounds which were produced by required culture of Fusariumoxysporum isolated from roots of citrus trees affected with roots not disease.Velmurugan et al (2009), extracted water soluble pigments from *Monascuspurputreus, Isariaspp, Emericellaspp, Fusariumspp* and *Penicilliumspp* and optimized the process parameters of dyeing leather. Shirata et al(2000) isolated Janthino bacterium lividum from wet seek thread and grew it n wakimoto medium to yield a bluish purple pigment identified as a mixture of violacein and deoxyviolavein.

Studies have also been carried out to intensify the rate of pigmentation to increase the colour yield by incorporating carbon and nitrogen sources in the growth media.

The purpose of this research is to enhance colour yield by using fungal strains of *Trichoderma, Alternaria and Curvularia lunata* on different growth media supplementing it with carbon and nitrogen sources.

2. Materials And Methods

2.1 Fungal species

Three species that is *Trichoderma pseudo kiiniipii*, *Alternaria alternate* and *Curvularia lunata* were sourced from Division of Plant Pathology, IARI.

2.2 Fermentation Medium

(i) **Standard mediums**

PD Broth, MPI, MPII, Basal medium, SBM, GA and Defined medium.

(ii) **Natural sources of medium**

Rice water, Wheat flour water and Potato water.

(iii) Waste sources of medium

Rice water procured from canteen/dhaba, rotten banana and Potato peel.

2.3 Fabric

Silk and wool

2.4 Chemicals used

Carbon sources

Dextrose, Sucrose, Fructose, Starch

Nitrogen sources

Sodium nitrite,Aspergine, Glutamic acid, Beef extract and Ammonium sulphate.

Other used in scouring, degumming and dyeing.

2.5 Equipment

Spectrophotometer, Autoclave, Incubator, Laminar chamber and Homogeniser.

Experimental

(A) Pigment Production in Different Fermentation Medium

The three species *Trichoderma pseudo kiiniipii*, *Alternaria alternata* and *Curvularia lunata* were inoculated on PD slants and subsequently in PD Broth medium. The mycelium and broth were subjected to different methods of screening that is only filtrate, homogenizing method. Temperature, time and static or shaking conditions were optimized before checking the optical density.

Thereafter all the three fungi sp were inoculated on other mediums that is MPI, MPII, BASAL medium, SBM and defined medium in static condition to see any occurrence of pigment production.

In the next phase 4 carbon sources namely sucrose, dextrose, fructose and starch and 5 nitrogen sources namely ammonium sulphate, beef extract, sodium nitrite, aspergine and glutamic acid were added in defined medium with three species and again cultured at 28˚C for 3weeks in static condition and their optical density was taken.

After that the three species were inoculated in natural sources of medium with optimized carbon source that is dextrose and nitrogen source that is ammonium sulphate for better pigment production at 28˚C in static culture and their optical density was taken.

To increasing the pigmentation properties different waste sources were tried with carbon source only.

3. Result And Discussion

3.1 ***Trichoderma*** on (PD) potato dextrose broth (28˚C) produced yellow color on wool and silk respectively, *Alternaria* produced a brilliant golden brown on wool and silk respectively and *Curvularialunata* produced a skin (melanin) color on wool and silk respectively.

Table 3.1 Optical density of pigment produced by *Trichoderma, Curvularia*and*Alternaria* at potato dextrose medium& optimum temperature

Fungal species (28˚ C)	**O.D Of Color Produced According To Days At 410nm**							
	0	5	10	15	20	25	30	35
Trichoderma	-	0.180	0.199	0.380	0.525	0.710	0.785	0.785
Curvularia	-	0.350	0.490	0.640	0.919	0.920	0.990	0.990
Alternaria	-	0.360	0.610	0.700	0.820	0.950	0.985	0.985

3.2 In MPI, MPII, BASAL, GA and DEFINED MEDIUM-*Trichoderma* did not produce any color. In SBM medium *Trichoderma* changed the color into pinkish red tone from yellow on wool and silk respectively.*Curvularialunata*and *Alternaria* produced no color in any of these medium.

Table 3.2 Optical density of pigment produced by *Trichoderm,* at defined medium with carbon and nitrogen sources and at rice water medium with carbon sources& optimum temperature

Sources (carbon & nitrogen)	O.D OF COLOR PRODUCED BY TRICHODERMA ACCORDING TO DAYS AT 410nm(28 ˚C)							
	0	5	10	15	20	25	30	35
Sucrose	-	-	-	0.120	0.198	0.230	0.300	0.300
Dextrose	-	-	0.330	0.339	0.489	0.589	0.670	0.670
Starch	-	-	0.128	0.329	0.560	0.867	1.20	1.20
Fructose	-	-	-	0.239	0.340	0.367	0.389	0.389
Sodium nitrite	-	-	0.170	0.220	0.310	0.450	0.469	0.469
Aspergine	-	-	0.200	0.220	0.390	0.399	0.430	0.430
Glutamine	-	-	0.190	0.250	0.300	0.397	0.467	0.467
Beef extract	-	-	-	-	-	-	-	-
Dextrose + rice water	-	0.167	2.89	2.89	2.89	2.89	2.89	2.89
Dextrose + waste rice water	-	0.167	2.89	2.89	2.89	2.89	2.89	2.89
Sodium sulphate	-	-	0.140	0.230	0.310	0.490	0.577	0.577

3.3 *Trichoderma* in defined medium with sucrose, dextrose, starch and fructose produced light yellow color on wool and silk respectively, *Alternaria* and *Curvularia lunata* produces no color on wool and silk. *Trichoderma* on DEFINED MEDIUM with Ammonium sulphate produced color on wool and silk respectively. *Alternaria* and *Curvularia lunata* produced no color. *Trichoderme sp*. is observed to produce color. *Alternaria* and *curvularia lunata* was eliminated. *Trichoderma* on rice water with carbon source produced better color as compare to nitrogen

source. No color was produced in wheat flour and potato water medium. With the ratio of carbon and nitrogen source *Trichoderma* produced no color. *Trichoderma* on rice water (waste source) with carbon source produced brilliant yellow color on wool and silk respectively. No color was observed in rotten banana and potato peel water medium.

Table 3.3 Percentage absorption of silk and wool dyed with *Trichoderma*

S.No.	Textiles Fabric	*Trichoderma* rice water(28˚C)		
		O.D Before Dyeing	O.D After Dyeing	Percentage Absorption(%)
1	Silk	2.76	0.425	84.08
2	Wool	2.67	0.267	90

3.4 The percentage absorption of wool is more than silk samples for *Trichoderma* respectively. Samples were also visually examined and found to have darker shade on wool than silk. The amorphous regions of fiber determine the penetration of dye molecule into the fiber. Since wool has more amino acids and higher amorphous area than silk, absorbency of wool is greater than that of silk.

It was found that the percentage absorption of wool is more for dye from *Trichoderma*. The wool fiber contains equal amount of amino and carboxyl groups which ionize and form zwitter ion. At low pH the hydrogen ions leaving behind ionized groups. Thus wool absorbs maximum dye in acidic medium. (Mathur and bhandari,2001)

Table 3.4 K/S, L*,a*, b* values of final dyed samples of silk and wool with *Trichoderma*

S.NO.	Textile Fabric	*Trichoderma*rice water with dextrose(28˚C)			
		K/S	L*	a*	b*
1	Silk	18.11	16.034	-1.608	33.897
2	Wool	32.38	12.217	4.103	35.738

3.5 Wool has higher K/S value than silk, hence wool has higher color depth than silk. Results indicate that wool dyed with *Trichoderma* has higher K/S value. The a* value of *Trichoderma* dyed silk sample is in negative thus indicating green component whereas the a* value of wool dyed sample is positive thus indicating magenta component. The b* value of both the samples is in positive thus indicating yellow component.

Table 3.5 Rub and wash fastness tests of final dyed samples of silk and wool dyed with *Trichoderma*

S.NO.	Textile Fabric	Rub Fastness		Wash Fastness		
		Dry	Wet	Color change in specimen	Staining on standard fabric 1	Staining on standard fabric 2
1	*Trichoderma (silk)*	5	5	5	5	5
2	*Trichoderma (wool)*	4	4	5	4	4

3.6 Fastness to rubbing of the samples as per table depicted that the dry rub fastness of silk *Trichoderma* is excellent i.e. no staining was recorded. The wet rub fastness of silk dyed sample, was recorded as 5 i.e. no staining so have excellent wet rub fastness .the dry and wet rub fastness of wool was recorded as 4 i.e. less staining was recorded so it have good to very good fastness rub fastness. Fastness to perspiration of the samples depicted that in alkaline as well as acidic condition perspiration fastness of silk *Trichoderma* is excellent while in other hand wool has poor to good perspiration fastness.The rating given in table is as per standard SDC grey scale

Table 3.6 Perspiration fastness test of final dyed samples of silk and wool with *Trichoderma*

S.No.	TEXTILE FABRIC	ALKALINE CONDITON			ACIDIC CONDITION		
		Color change in specimen	Staining on standard fabric 1	Staining on standard fabric 2	Color change in specimen	Staining on standard fabric 1	Staining on standard fabric 2
1	*Trichoderma (	5	5	5	5	5	5

	silk)						
2	***Trichoderma (wool)***	3	3	3	3	3	3

3.7 To optimize the quantity of starch for maximum pigment production starch of the rice water medium was quantified with the help of spectrophotometer. That enables the standardization of the medium for replicating the study in the future.

Table 3.7 Light fastness tests of dyed samples of silk and wool dyed with *Trichoderma*

S.NO.	**Textile Fabric**	**Test Method**	**Test Result**
1	***Trichoderma* silk**	**Manually**	*****
2	***Trichoderma* wool**	**Manually**	****

3.8 The rice water contains 0.2 % of starch in 20 ml of liquor in which maximum pigment had produced.

Table 3.8 Optical density of the rice water compared with potato starch

S.NO.	**MEDIUMS**	**Quantity of starch in 20 ml of water with 1 drop of iodine**					
		0.05%	**0.1%**	**0.2%**	**0.3%**	**0.4%**	**0.5%**
1	**Starch**	0.169	0.323	0.580	0.789	0.868	1.06
2	**Rice water**	0.460	0.541	0.580	0.580	0.868	1.06

Conclusion

Our study showed that the natural source of medium that is rice water with carbon source gave the best result and this is the cheapest source of medium because the rice water procured from dhaba/ canteen. The colour fastness that is wash fastness, rub fastness, perspiration fastness (measured according to SDC grey scale) of silk was better than wool. The quantified starch value is 0.2% in which pigment was produced.

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The authors have shared their experiment and the outcome