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PIGMENT EXTRACTION METHODS FROM FUNGI FOR INDUSTRIAL APPLICATIONS: A REVIEW

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ABSTRACT

This review aims at finding pigment extraction methods that can be employed in various industries like textiles, wood, leather and primarily food industry. Color is the prime feature of any food product as it enhances its appearance and acceptability. Several synthetic dyes are available that causes severe heath problems. Hence pigments produced from natural resources are gaining worldwide popularity. In addition to this the availability of cheap raw materials, their supply throughout the year and antibacterial and antifungal properties of some pigments have added to their value. Many lower and higher fungi are reported as potential sources of pigments like carotenoids, flavonoids, xanthophylls, anthraquinones, etc. These pigments are easy to extract through microbial fermentation through Mineral-salts basal media or PDA and a variety of agro—industrial wastes serving as raw material.

Keywords: antibacterial, antifungal properties, extraction methods , fungal pigments, mineral- salts basal media.

I. INTRODUCTION

Among the various ever increasing applications of biotechnology in industries, dyeing of fabrics, leather and wood has found a prime importance. A world wide interest has surfaced in development of procedures / extracting techniques for the production of various pigments from natural sources such as plants, animals, cell-cultures, tissues, etc.[1,2,3]. Scientists are continuously being pressurized to turn towards natural pigment producers due to the increasing safety hazards associated with many artificial synthetic colorants being widely used in foodstuff, cosmetic and pharmaceutical industry. Micro-organisms are now also being applied to produce drug compounds in presence of natural substrates such as milk and whey so as to reduce the harmful effect of chemical synthesis on environment[4]. Pigments to be extracted from micro- organisms is advantageous as although there are a number of natural pigments maximum are being produced through plants and hence are not available in sufficient quantities [5

In food industries they are used as additives, colour intensifiers, antioxidants, etc., textile industries used for textile dyeing and also in the manufacturing of cloths with antimicrobial properties for example anthraquinones. Pigments come in a wide variety of colours and some are water-soluble. For these reasons, many of these

compounds have been produced, isolated, and characterized. Another industrially important feature of microbial pigments is colorfastness against washing and crocking (color loss due to rubbing). This key feature can contribute much towards the already pollutant laden textile industry and can reduce the environmental impact of textile finishing industry. Before a couple of decades, fungal pigments were being used for wood staining, during the last decade, however, a novel interest has surfaced in employing such pigments for direct dyeing textiles [6]. On the contrary, synthetic dyes, which became popular due to their colorfastness, availability in a wide range of colors, low production cost, etc. are a potential source of various disorders, skin allergies and also cancer. In addition to this, the synthetic dyes generate ample amount of hazardous waste and green house gases during their processing [7,8,9].

This review article enlightens such pigments that can be obtained from naturally occurring fungi and also the extraction technique associated with them.

II. VARIOUS PIGMENTS PRODUCED FROM FUNGI.

The most significant natural pigments are carotenoids, flavonoides, tetrapyrroles, and some xathophylls as astaxanthin, etc. Some of these natural colorants are used in energy drinks, baby foods, processed cheese, breakfast cereals, , pastas, , fruit drinks, sauces and some vitamin- enriched milk products. Fungi are reported as potent producing micro-organisms [10,11,12] . Pigments like anthraqiunone, anthraquinone carboxylic acids, pre-anthraquinone are extracted from filamentous fungi [13]. For dyeing of leather Velmurugan et.al have extracted pigments from Monoascus purpureus, Isaria spp., Emericella spp, Fusarium spp, and Penicillium spp.[14]. High yielding pigmens have also been reported to be extracted from from fungi belonging to genus Paecilomyces, producing red, yellow pigments, genus *Aspergillus* and *Penicillium*. Among these those pigments extracted from genus *Monoascus* and *Penicillium* have a potential use in food industry as they are not associated with critinin production [15, 16, 17].

In addition to the above reported pigments, various others are still under process of extraction. Such pigments are summarized in Table1.

III. ISOLATION OF FUNGAL STRAINS.

Many researchers believe that occurance of a variety of micro-oragnisms largely depends upon the natural environment from which they are isolated. Hence scientists like Velmurugan et al. have opted for high altitude and biodiversity hot-spot, The Nilgiris for their sample collection [17].

For direct dyeing of textiles Hinsch and Robinson collected samples from a rotting hardwood. In addition, domestic fridge, area surrounding a research laboratory, milking industry and oil refinery can also serve as good sites by providing adept fungal strains [18]. The pigment providing mushrooms were collected from their natural habitat.

All the samples were then cultured in PDA (Potato Dextrose Agar) using serial dilution and then pure cultures were obtained. The media was supplemented with antibiotics to prevent bacterial growth.

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IV. CULTURE, EXTRACTION AND PURIFICATION OF PIGMENTS.

4.1 *Culture Media* – The routine culture media for fungal cultivation is PDA, however, some researchers have also employed Mineral salts-glucose medium for the extraction of fungal pigments. Certain industrial by-products of agro- industrial origin have also shown their potential in production of a variety of pigments. These industrial by- products are extremely hazardous to environment if introduced untreated into it. Hence conversion of such wastes to materials such as raw substrate for pigment production can reduce their impact on environment and also would provide financial benefits. Table 2 summarizes such wastes which have been employed by various researches for economic pigment extraction.

4.2 *Culture conditions*- Pigments are derived from biosynthetic pathways such as the shikimate pathway, acetate- malonate pathway and the melvalonate pathway [19] **.** Hence the culture conditions are made such that to assist in such pathways and the pigment yield could be increased. Most of the researchers have followed the procedures of Nagia and El- Mohammady, in which mycelia discs are cut using cork borer of definite diameter and inoculated in Potato –Dextrose broth or Mineral- salts glucose medium [20]. Depending upon the time required for pigment production the flasks are kept in a BOD incubator at 27-30° C for 4-6 weeks. Also a short change in pH and temperature can increase the yield of pigments in *Penicillium* species(Mendez.,et.,al,2011). After this, the mycelia are harvested and filtered using Whatman's filter paper [17,21].

4.3 *Pigment extraction*- The most common pigment extraction method involves addition of 95% ethanol (v/v) in a two stage process [17,21]:-

i. In the first stage about 60% of the total solvent was added to the supernettant and kept at 30° C for 30 minutes in a rotary shaker at 180 rpm.

ii. After 30 minutes the ethanolic mixture is centrifuged for 15 minutes at 3700-3800 rpm to remove any residual mycelia and centrifuged again for 5 minutes at 3790 rpm. The supernettants were collected and filtered through Whatmann's filter paper and the filterate was kept for further analysis.

In an alternative method Hinch and Robinson(2016) applied Dichloromethane (DCM) for pigment extraction from fungal agar plates. The parafilm was removed from agar plates 48 hrs prior to extraction. The plates were opened and kept in fumed hood to allow the mycelia to dry out. After drying the mycelia were cut into small pieces of approximately 2 cm by hands and crushed mycelia were placed in a glass round bottomed flask. To this flask 150 ml DCM was added along with an octagonal magnetic stirrer. To prevent evaporation of DCM a rubber stopper was placed on top of flask and the flask was transferred to a stir plate and stirred for 30 minutes at 230 rpm. The contents were then stirred and filtered through Whatmann's filter paper to obtain the dye. For obtaining sufficient quantity of dye the process was repeated as per requirement [18].

4.4 Analysis of pigment- The pigments thus obtained are then analysed using spectrophotometer with appropriate wavelengths and FTIR.

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1.TABLES

Table 1. Pigments of industrial importance from various fungi (Blanc.,et.,al,1994, Mashiro.,et.,al, 1994, Marova.,et.,al 2012).

Pigment	Color	Fungi	Status
Ankaflavin	Yellow	*Monoascus spp*	IP
Anthraquinone	Red	*Penicillium candidum*	IP
Monoascorubramine	Red	*Monoascus spp*	IP
Rubropanctatin	Orange	*Monoascus spp*	IP
Lycopene	Red	*Fusarium sporotrichoides*	IP
Melanin	Black	*Sacchromyces neoformis*	RP
Napthoquinone	Deep blue red	*Cardyceps unilateralis*	RP
β-carotene	Yellow-orange	*Fusarium sporotrichoides*	RP
β-carotene	Yellow-orange	*Phycomyces blacksleeanus*	RP
Unknown	Red	*Paecilomyces sinclairii*	RP
Astaxanthin	Pink-red	*Xanthophyllomyces dendrohous*	DS
Torularhodin	Orange-red	*Rhodotorula sp*	DS
Unknown	Red	*Monoascus purpureus*	RP
Unknown	Yellow	*Penicillium spp*	RP
Unknown	Pink	*Emericella spp*	RP
Agaricone	Yellow	*Agaricus xanthodermus*	IP
Muscopurpurin	Orange-red	*Amanita muscaria*	IP
Unknown	Ornage-red	*Paecilomyces farinosus*	RP

Notes: Industrial production: IP. Research project stage: RP. Developmental stage: DS.

Table 2. Various substrate used and pigment associated with them.

S.no	Substrate	Micro-organism	Pigment extracted	Reference
1	Whey	*R.glutinis*	β-carotene	[22]
2	Mustard waste	*X. dendrohous*	Astaxanthin	[23]
3	Plant extracts.	*X. dendrohous*	Astaxanthin	[24] [25]
4	Chicken feathers.	*R. glutinis*	Carotenoids	[26]
5	Rice bran	*M. purpureus*	Monascorubramine	[27]
6	Corn cob substrate	*M.purpureus*	Monoascorubramine	[17]
7	Mariegold flower	*R. glutinis*	Luteins	[28]

8	Durian seed	*Monoascus spp.*	Angkak moncolin	[29]
9	Kinnow waste	*M. purpureus*MTCC369	Bio- pigment	[30]
10	Sugarcane bagasse	*P. echinulatum*9A02S1	Cellulose, Xylanase	[31][32]

V. CONCLUSION

The overall objective of this review was to explore the potentially active pigment producing fungi that are widely occurring naturally and can provide excellent dyes for industrial purposes without damaging our planet. Furthermore some of these pigments have also been reported to possess certain antibacterial and antifungal properties that add to their valuability towards mankind and livestock.

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REFERENCES

[1]. Dziezak, J.D. (1987) "Applications of food colorants". *Food Technology 41, 78–80.*

[2]. Francis, F.J. (1987) "Lesser-known food colorants". *Food Technology 41, 62–68.*

[3]. Francis, F.J. (1989) "Food colorants: Anthocyanins". *Critical Review in Food Science 28, 273–313*.

[4]. Shrivastava ,M., Saraf A, Shukla K.K and Sharma D. "Isolation of Bioactive Metabolites Producing Fungal Strains From Milk Products and Milk Industries of Raipur District." *World Journal of Pharmacy and Pharmaceutical Sciences. 6(9), 1652-1658.2017*

[5]. Lauro, G.J. (1991)" A primer on natural colors". *Cereal Foods World 36, 949–953*.

[6]. Osman, M. Y., Sharaf, I. A., Osman, H. M., El-Khouly, Z. A., & Ahmed, E. I. (2004). "Synthetic organic food colouring agents and their degraded products: Effects on human and rat cholinesterases". *British Journal of Biomedical Science, 61, 128–132.*

[7].Hamlyn, P. F. (1995). The impact of biotechnology on the textile industry. *Textile Magazine, 95, 6–10.*

[8]. Rai, H., Battacharyya, M. S., Singh, J., Bansal, T. K., Vats, P., & Banerjee, U. C. (2005). Removal of dyes from the effluent of textile and dyestuff manufacturing industry– A review of emerging techniques with reference to biological treatment. *Critical Reviews in Environment Science and Technology, 35, 219–238.*

[9]. N. Nagpal, *et al.*, "Microbial pigments with health benefits-A mini review," *Trends in Biosciences, 4,. 157-160, 2011*.

[10]. Venil C.K., ,Zakaria, Z.A., Ahmed W.A (2013) "Bacterial pigments and their applications," *Process Biochemistry*,vol. 48, *pp. 1065-1079.*

[11]. L. Dufossé, "Microbial Production of Food Grade Pigments," *Food Technology & Biotechnology*, vol. 44, 2006.

[12]. Babitha, S., Soccol, C. R., & Pandey, A. (2007). Effect of stress on growth, pigment production and morphology of Monascus sp. in solid cultures. *Journal of Basic Microbiology*, 47, 118–126.

[13]. Mapari, S.A.S., Hansen, M.E., Meyer, A.S., Thrane, U., 2008a." Computerized screening for novel producers of *Monascus* like food pigments in *Penicillium* species." *J. Agric. Food Chem.*, **56**(21):9981-9989.

[14]. Velmurugan P, Hur H, Balachandar V, Kamala-Kannan S, Lee KJ, Lee SM, Chae JC, Shea PJ, Oh BT (2011): Monascus pigment production by solid-state fermentation with corn cob substrate. *Journal of Bioscience and Bioengineering* 2011, 112:590-594.

[15]. Blanc, P.J., Loret, M.O., Santerre, A.L., Pareilleux, A., Prome, D., Prome, J.C., Laussac, J.P., Goma, G., 1994. "Pigments of *Monascus*." *J. Food Sci.*, **59**(4):862-865.

[16]. Masahiro, K.O., Mine, K., Taya, M., Tone, S. and Ichi, T. (1994) "Production and release of anthraquinone pigments by hairy roots of madder (Rubia tinctorum L.) under improved culture conditions." *Journal of Fermentation and Bioengineering 77, 103–106.*

[17]. Velmurugan P. et.al (2010): Natural pigment production from five filamentous fungi for industrial application and dyeing of leather. *Carbohydrate polymers:* 79: 262-268.

[18]. Hinch E.M and Robinson S.C (2016) " Mechanical Color Reading ofWood-Staining Fungal Pigment Textile Dyes: An Alternative Method for Determining Colorfastness." *Coatings,6, 25*.

[19]. Velišek J., Cejpek K. (2011): Pigments of higher fungi – a review. *Czech Journal of Food Sciences*., 29: 87–*102.*

[20]. Nagia, F.A., El-Mohamedi, R.S.R. (2007). *Dyes and Pigments,* 75(3): *550 555*.

[21]. Sharma D., Gupta C, Aggrawal S., Nagpal D.(2012): "Pigment extraction from fungus for textile dyeing." *Indian Journal of Fibre and Textile Research:* 37: 68-73.

[22]. I. Marova, *et al.*, "Use of several waste substrates for carotenoid-rich yeast biomass production," *Journal of Environmental Management*, vol. 95, pp. S338-S342, 2012.

[23]. J. Tinoi, *et al.*, "Utilization of mustard waste isolates for improved production of astaxanthin by *Xanthophyllomyces dendrorhous*," *Journal of Industrial Microbiology and Biotechnology*, vol. 33, pp. 309-314, 2006.

[24]. Stachiowaik B,(2012) "Astaxanthin synthesis by yeast *Xanthophyllomyces dendrorhous* and its mutants on media based on plant extracts." *Indian Journal of Microbiology." 52(4), 652-659.*

[25]. Kim S.K, Lee J.H, Lee C.H, Yoon Y.C.(2007)., "Increased carotenoid production in *Xanthophyllomyces dendrorhous* G276 using plant extracts," *Journal of Microbiology-Seoul-*, 45, 128-132.

[26]. M. Taskin, *et al.*, "Use of waste chicken feathers as peptone for production of carotenoids in submerged culture of *Rhodotorula glutinis* MT-5," *European Food Research and Technology*, vol. 233, pp. 657-665, 2011.

[27]. Babitha, S.: Microbial pigments, pp. 147–150, in: Nigam, P.S. and Pandey, A. (Eds.), Biotechnology for agro-industrial residues utilisation, vol. 5. Springer, Heidelberg (2009).

[28]. Bhosale, P., & Gadre, R.V.,(2001) "Production of Carotene on tagetes flower by Rhodotorula glutinis." *Applied Microbiology and Biotechnology* 55, 423-427.

[29]. Srianta I, Novita Y, Kusumawati N, (2012) " Production of monascus pigments on durian seed: effect of supplementation of carbon source". *Journal of Pure and Applies Microbiology*, 6:59-63.

[30]. Panesar R (2014) "Bioutilization of kinnow waste for the production of biopigments using submerged fermentation." *International Journal of food And Nutrition Sciences, 3:9-13.*

[31]. M. Camassola and A. J. P. Dillon (2007), ―Production of cellulases and hemicellulases by *Penicillium echinulatum* grown on pretreated sugar cane bagasse and wheat bran in solid-state fermentation,‖ *Journal of Applied Microbiology*, 103(6), 2196–2204.

[32]. Sayyed. I and Majumdar D.R. (2015): Pigment production from fungi. *International Journal of Microbiology and Applied Sciences.* Special issue-2:103-09.