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Microbial Pigments for Food Coloring and Textile Dyeing: a Review

Asefa Keneni

Department of Biology, College of Natural and Computational Sciences, Ambo University, P.O.Box 19, Ambo, Ethiopia. E-mail Address: asefakeneni@yahoo.com

Abstract

At present, demand for natural sources of coloring substances is increasing as a result of increasing consumer's awareness for the healthy safety of chemically synthesized pigments used in different sectors. These natural coloring substances has been obtained from plants (fruits, vegetables, roots, and flowers); animals such as scale insects and microorganisms. Extraction of natural colors from plants have many disadvantages including limited supply in the season of bad climatic conditions, batch to batch variation and instability under some environmental conditions. However, from all sources of natural pigments, microorganisms are having great potential, since the productivity will be increased by fermentation process development and the microorganisms are amendable to genetic engineering. Different group of microorganisms (bacteria, yeasts, fungi and micro algae) have been observed to produce different pigments with different chemical structures which are having different application. These pigment molecules include: carotenoids, melanin, flavins, quinines, prodigiosin and more specifically monascin, violacein, or indigo. These microbial pigments are having application in the field of pharmaceutical, cosmetics, food/feed, textile, agriculture and farming. Prodigiosin, violacein and some carotenoids have also receiving increasing interests since these microbial metabolites have valuable application as immunomodulating, anticancer and bioactive molecules. The rest of the carotenoids and fungal polyketides are valuable microbial metabolite used as food/feed additives or food coloring agents. In Ethiopia, very few literatures are available on microbial pigment production and application as food colorant and textile dyeing. In order to isolate and characterize pigment producing microorganisms from a local habitat and formulation of pigments for utilization, information about the pigment producing microorganisms is required. Therefore, this paper reviews microbial sources of pigments and their uses in food/feed and textile dyeing.

Keywords: Carotenoids, Food coloring, Microbial pigments, Polyketides, Textile dyeing

Introduction

Pigmented microorganisms producing pigments with different chemical structures and color are ubiquitous in nature. These belong to all the major groups of microorganisms (Dufosse 2006). Among the common pigment molecules produced by microbial species are carotenoids, melanin, flavins, quinines, prodigiosin and more specifically monascin, violacein, or indigo (Dufosse 2006). At present microbial pigments holding an enormous range of biological activities are useful high value products and applied in the field of pharmaceutical, cosmetics, food/feed, textile, agriculture and farming (De Santis *et al.,* 2005; Martinkova *et al.*,1999). In addition, with the increasing awareness of toxicity of synthetic colors, the demand for pigments from natural sources has increased (Babu & Shenolikar, 1995). These natural colors are generally extracted from fruits, vegetables, roots and microorganisms and are often called "biocolors" because of their biological origin and have proved to be safe and edible coloring agents (Babu & Shenolikar, 1995). Due to the global increase in the manufacture of processed foods and the continued consumer demands for natural food ingredients, the market for natural colorants for food use is estimated to grow (Mapari *et al.*, 2010). Chemically two major groups of food pigments are characterized from bacteria, microalgae, yeasts and fungi: carotenoids and polyketides. The other sector which uses coloring agent is textile industry. Almost all textile dyes originated from chemical synthesis, which are allergic and or carcinogenic, and the produced colors are non biodegradable as well. As a result, biological sources of the textile coloring substances are required. Besides bioactive microbial pigments like prodigiosin and violacein have been given priority as they are found to have potential industrial application as anticancer and immunomodulating agents (de Araújo *et al.*, 2010). Molecular structures of different microbial pigments are shown in Figure 1. The different microbial pigments, the producing group(s) of microorganisms and their function(s)/application of these pigments are presented in Table 1.

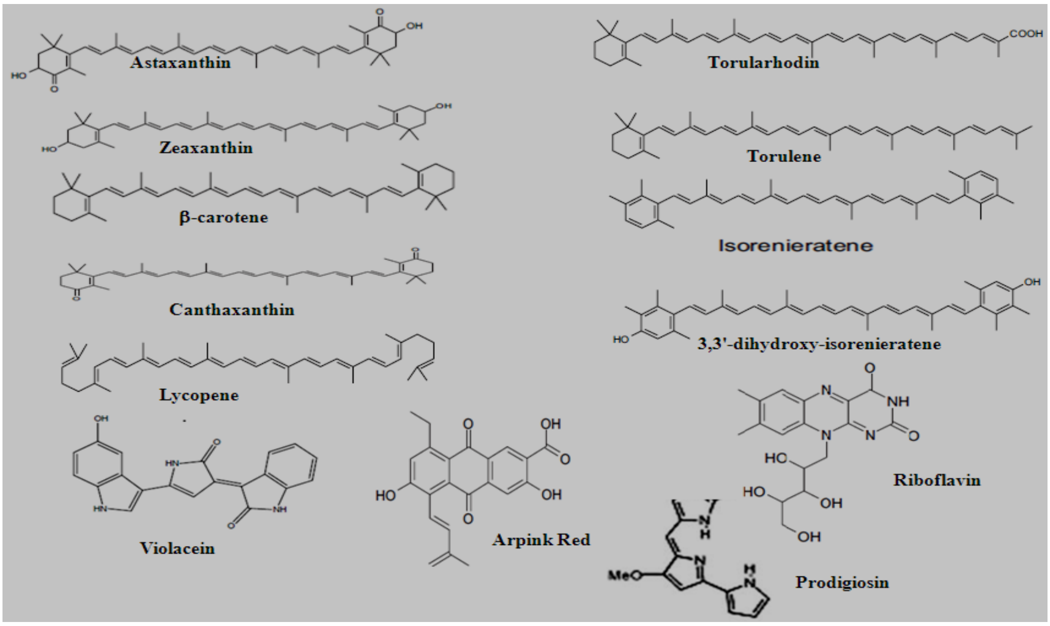

Figure 1. Molecular structure of different microbial pigments (de Araújo *et al*., 2010; Dufosse 2006).

Table1. Different microbial pigments, producing microbial group(s) and the possible function(s) or application(s) of these pigments

Pigment group (type)	Producing microbial group	Possible function(s)/application
Carotenoids: β-carotene, astaxanthin, anthraxanthin,α-carotene, canthaxanthin, β-Cryptoxanthin, fucoxanthin,Lutein, Violexanthin,, neoxanthin, zeaxanthin	Archeae, bacteria, yeasts, fungi, microalgae	Pro-vitamins, food additives/ food coloring, anti aging, protect against degenerative diseases;
Prodigiosins	Bacteria	Immune modulating, anti cancer, antibacterial, anti fungi, anti protozoan etc.
Violacein	Bacteria	Bioactive
Polyketides: anthraquinones, naphthoquinones and azaphilone)	hydroxyanthraquinones,	Filamentous fungi | Food additives/food coloring agent, food preservatives and textile dyes etc.

Sources: De Araújo *et al*., 2010; Babu & Shenolikar, 1995; De Santis *et al.,* 2005; Dufosse 2006; Mapari *et al*. 2010

Production and application of Bio-pigments in Ethiopia

Like other parts of the globe, Ethiopians` through the ages has been using coloring substances for food, textile products, leather products, pharmaceutical processing, printing inks and cosmetics. (Before the advent of the chemically synthesized coloring substances and still today, Ethiopians have been using natural sources of coloring substances mainly obtained from plants. Still today, in Ethiopia red pepper (*Capsicum* spp) is added to wot as condiments as well as to give red color) to the product which is commonly known as (keyi wot) “keyi” means red and “irdi” (*Curcuma spp*) is added to impart yellow color to “wot” which is commonly called “alicha”. This is still continuing and healthy in homemade foods. But in all non food sectors, at present, the sources of coloring substances are chemically synthesized (Walford J 1980). The increased awareness of the healthy effect of synthetic sources of coloring substances called for the replacement or supplement of the chemical sources of the coloring substances with those originated from biological sources. Nature is rich with materials that can be used as sources of colored substances (flowers, fruits, roots, pigmented leaves and microorganisms).

Microbial product production and formulation is not yet taken as common trend in Ethiopia. But “beer” one of the microbially produced product has been produced by imported strains of yeast (*Saccharomyces cervisiae*) and baker`s yeast “irsho” (*Saccharomyces cervisiae*). Even though, other microbial secondary metabolite including pigments is not hitherto studied well in Ethiopia, three isolates of red pigment producing bacterial strains were isolated from soils collected from different localities of the Ambo University main campus, Western parts of Ethiopia, and all three were characterized and identified as *Serratia* species and the pigment produced as prodigiosin (Asefa Keneni 2011). Prodigiosin is, one of the microbial bioactive compounds having an application as anticancer and immunomodulating molecules (de Araújo *et al*., 2010).

Food grade microbial pigments are not isolated, characterized, identified, formulated and applied in Ethiopia. Picture 1 show the diversity of pigmented fungi isolated from plant samples collected from Chelemo forest Western Shoa zone, West part of Ethiopia. Pigment production from microorganisms is a potential untouched area of research for microbiologists and natural product scientists.

Figure 2. Pigmented microorganisms isolated from plant samples collected from Chelemo forest (Western Shoa, Western part of Ethiopia), grown on a surface of agar plate by the Author.

Why microorganisms produce pigments?

Though the exact biological function of microbial pigments in the producing microorganisms is not yet clearly understood, in higher organisms these play vital roles in sensing the environment to guide their interactions with other organisms (Hill, 1999). However, as microorganisms lack color perception, it is plausible to assume that evolutionary selective pressures behind the acquisition of pigments somehow promotes survival of the producing organisms.

With help of recent advances in biotechnology, molecular biology and recombinant DNA technology, contemporary researchers are analyzing the molecular genetics and biochemical basis of microbial pigmentation in microbial pathogenesis. Investigations using purified pigments or isogenic mutants with altered pigmentation have begun to reveal how these molecules can provide a survival advantage for the pathogen in the host environment and/or produce significant alterations in host cells and immune response pathways (Williamson *et al*., 2007). The data generated with this regard has proposed some natural functions for microbial pigments, such as protection against ultraviolet radiation (Romero-Martinez *et al*., 2000), oxidants (Liu *et al*.,2005), extremes of heat and cold (Paolo Jr *et al*., 2006), serve as antimicrobial compounds (van Duin *et al*.,2002; Baron & Rowe, 1981), and helps in the acquisition of specific nutrients and energy by photosynthesis such as in cyanobacteria (Chatfield & Cianciotto, 2007). Though in the non pathogenic microorganisms the exact biological function of pigmentation is not known, pigment production has been suggested to provide survival advantage in the environment than the non pigmented strain of the same microbial species (Demain, 1986).

Microbial pigments and other secondary metabolites (idiolites) are special metabolites usually possessing peculiar chemical structures and are produced as secondary metabolite as onset of their productions often correlates with the ending of the most active growth phase (trophophase) and the beginning of the stationary phase or 'idiophase' (Vining, 1992).

Limitation of contemporary sources of pigments

Available evidences showed extensive use of natural colorants, such as plant extracts and wine, to color foods that was adopted as early as 1500 BC Meggos (1995).The historical use of natural colorants was taken over by chemically synthesized colors at the middle of 19th century (Downham & Collins, 2000). The industrial revolution had resulted in widespread use of different colorants both in the food industry and processed foods. Starting from the beginning of the 20th century, chemically synthesized colors were derived from Aniline- a petroleum product (Downham & Collins, 2000). This development was primarily governed by their easier and more economical purification and superior coloring properties. However, safety concerns that emerged with increasing application of these synthetic coloring agents, led to numerous regulations throughout the world, resulting in the revival of the demand for natural food colorants (Downham & Collins, 2000; Babu & Shenolikar, 1995). Presently, consumer awareness has been increasing over the link between diet and health; as a result, the trend emerged towards clean label ingredients (Mapari *et al.*, 2010). Natural food colorants that are currently authorized in the European Union (EU) are mostly derived from the raw materials obtained from flowering plants (antocyanine and beta carotene and insects, as in the case of carminic acid derived from scale insects (Allevi *et al.*, 1998). The current production of natural colorants is dependent on the external, seasonal supply of raw materials, potentially resulting in batch-to-batch variations in the extracted pigment profile (Spears 1988). As natural colorants are extracted from natural sources, they are, in most cases, mixtures of varying composition that depends upon the cultivar and climatic conditions and, therefore, not easy to characterize with respect to purity and contaminants. The extracted plant pigment mixtures are not likely to be produced as pure compounds or well-defined pigment compositions, unless they are produced in plant tissue cultures; nevertheless, obtaining high yields and longer cultivation periods could be major bottlenecks (Mapari *et al.*, 2010). Moreover, there is great variation in the stability and functionality of different classes of existing natural food colorants in extreme environments like change in temperature and pH (Mortensen 2006). This limits their application to certain types of processed food products that fit the stability requirements of the colorant (Mapari *et al.*, 2010). As a result, an alternative route being sought for the production of natural food colorants is through the application of biotechnological tools to microorganisms.

Microbial pigments as food coloring agents

Microalgae (Hejaz & Wijffel, 2004) and several classes of fungi and bacteria are known to produce a wide range of excreted water-soluble pigments, but the low productivity of algal cultures is a significant bottleneck for their commercialization (Wissgott & Bortik, 1996). Though use of fungi to color foodstuffs is not a novel practice; the use of *Monascus* pigments in food has been carried out traditionally in the Orient for hundreds of years (Tseng &Feldhein, 2001; Mapari *et al.,* 2010). Various food grade microbial pigments can be broadly classified chemically as polyketides and carotenoids (Mapari *et al.,* 2010; Lin *et al.,* 2008).

Fungal polyketides

Fungal polyketide pigments range in structure from tetraketides to octaketides having with four or eight C2 units (C-C, C=C) contributing to the polyketide chain. Representative classes of polyketides include the anthraquinones, hydroxylanthrax-quinones, naphthoquinones and azaphilone structures, each of which exhibits an array of colour hues (Mapari *et al.*, 2010; Lin *et al.,* 2008; Shier *et al.,* 2005). These polyketide pigments have specific significance in fungal taxonomic identification and species differentiation (Fabre *et al.,* 1993). These pigments are known to be produced by a number of fungal species, such as those belonging to the genera of *Aspergillus, Cordyceps, Culvularia, Epicoccum, Fusarium, Monascus, Paecilomyces, Penicilliu,* and *Roesleria* (Table2). However, *Monascus* species have been studied in more detail as source of food grade fungal pigments (Mapari *et al.,* 2006; Juzlova *et al.,* 1996; Blac *et al.*, 1994; Jongrungruangchok *et al.,* 2004).

Table 2: Safe Polyketides food grade pigments from different fungal species

Fungal species	Name of the pigment	Colour
Penicillim oxalicum	Anthroquinone	Red
P. aculeatum	Naphthoquinone	Red
P. pinophilum	Naphthoquinone	Red
P. purpurogenum	Naphthoquinone	Red
P. funiculosum	Naphthoquinone	Red
P. herquei	Naphthoquinone	Blue
Cordyceps unilateralis strain BCC 1869	Naphthoquinone	Red
Epicoccum nigrum	Orevctaene	Yellow
Culvularia spp	helmin thosporin	Maroon
Culvularia spp	cynodontin C	Bronze
Aspergillus spp	Viopurpurin	Purple black
Fusarium species	Bikaverin	Red
Fusarium spp	Nectria furone	Yellow brown
Roesleria hypogea	--	Green
Paecilomyces sinclaririi	--	Red

Sources: Mapari *et al*., 2010; Lin *et al.,* 2008; Mapari *et al.,* 2006; Shier *et al.,* 2005; Jongrungruangchok *et al.,* 2004; Juzlova *et al.,* 1996; Blac *et al*., 1994; Fabre *et al.,* 1993.

Monascus is often encountered in oriental foods, especially in Southern China, Japan and Southeastern Asia. Two species, *Monascus rubber* and *M. purpureus* are well investigated for production of food grade *Monascus* pigment, though many other strains have also been isolated around the world (Dufose, 2006). Currently, more than 50 patents have been issued in Japan, the United States, France and Germany, concerning the use of *Monascus* pigments in different foods (Dizon & Sanchez, 1984).

Annual consumption of *Monascus* pigments in Japan increased from 100 tons in 1981 to 600tons in 2000 and was valued at $1.5 million (Dufosse, 2006). In the fungus *Monascus*, ankaflavine and monascine are yellow pigments, rubropunctatine and monascorubrine are orange and rubropunctamine and monascorubramine are purple (Blanc *et al.,* 1994).

The coproduction of citirinin mycotoxin in association with different pigments by the *Monascus* fungi has however restricted the wide application of the *monascus* pigment, particularly in the Western World (Chen *et al.,* 2008). As a result identifying the safe food grade pigment producing fungi has remained an active area of research for more than a decade (Mapari *et al.,* 2006 & 2010). Fungal species producing save food grade polyketide pigments are presented in Table 2.

Carotenoids from microorganisms

Carotenoids are yellow to orange-red pigments that are ubiquitous in nature responsible for the pleasing yellow, orange and red colour of many foods, e.g., fruits, vegetables, egg yolk, some fish like salmon and trout, and crustaceans (Nelis &De Leenheer, 1991; Goodwin, 1980). Chemically they are composed of a polyene skeleton, which usually consists of 40 carbon atoms and is either acyclic or terminated by one or two cyclic end groups. The collective term 'xanthophylls' refer to substituted derivatives of carotenoids containing hydroxy-, keto-, methoxy-, epoxy- or carboxyl groups. Unsubstituted derivatives are commonly called 'carotenes' (Nelis & De Leenheer, 1991; Goodwin, 1980).

The best known function of carotenoids, such as α-carotene, β-carotene and δ-cryptoxanthin is to serve as provitamin A (Olson, 1989). In non photosynthetic bacteria carotenoids are also known to play a photoprotective role (Hausmann & Sandmann, 2000; Simpson, 1983). These photosensitive pigments are believed to act as a photoscreen, protecting vital macromolecules and cellular elements from the deleterious effects of UV and visible light. In more recent years, vitamin A-active and inactive carotenoids have also been found to have other beneficial effects on human health, for example enhancement of the immune system and reduction of the risk for degenerative diseases, such as cancer, cardiovascular diseases, macular degeneration and cataract (Nelis & De Leenheer, 1991; Olson, 1989; Hennekensc *et al*., 1986; Simpson, 1983; Marusichw & Bauernfeinjd, 1981; Krinskyn, 1979). Thus, carotenoids constitute one of the most valuable classes of compounds for application in pharmaceutical, chemical, food and feed industries.

Microbial carotenoids have attracted much attention in recent years, due to the possibility of increasing production by environmental and genetic manipulation (Kim *et al.,* 2006; Iturriaga *et al.,* 2005). The commercial utilization of microorganisms with biotechnological potential to produce carotenoids is presently limited by the high cost of production (Kim *et al.,* 2006; Lampila *et al*., 1985). However, the cost of carotenoid production by fermentation can be minimized by optimizing its process and using high pigment- producing microorganisms cultured in or on cheap industrial by-products as nutrient sources Kim *et al.,* (2006). There are different corotenoids produced from microbial sources for pharmaceutical and food and feed colouring agent (Nelis & Leenheer, 1991; Goodwin 1980). Of all the carotenoids, β- carotene and astaxanthin, are given more attention and mainly used in food, feed and pharmaceuticals industries.

β- carotene is produced primarily by fungi and yeasts and by some species of bacteria, algae, and lichens. At present the industrial production of β-carotene is by using the mould *Blakeslea trispora,* which is a plant commensal of tropical plants, some strains of which produce high levels of β-carotene. The highest yields have been obtained with a mixture of '+' and '−'strains of *B. trispora (*Sardaryan , 2004; Rouka & Mantzourdou, 2000). The fungus exists in '+' and '−' mating type, of which the '+' type synthesizes trisporic acid, a precursor of β-carotene. Mating the two types in a specific ratio, the '−' type then starts producing large amounts of β-carotene (Sardaryan, 2004).

Astaxanthin (3, 3′-dihydroxy-β, β-carotene-4, 4′-dione) is the principal carotenoides in marine animals, such as crustaceans, and salmonod. Astaxanthin provides attractive pigmentation to many farmed animals and contributes to consumer appeal in the market place. To obtain a natural red-pink colour the use of astaxanthin for pigmentation in aquaculture, especially as a feed supplement in farmed salmon and trout, is necessary, since animals lack the ability to synthesize it *de novo* (Kim *et al.,* 2006). This carotenoid imparts distinctive orange-red colouration to the animals. Astaxanthin also plays important metabolic functions in animals, which include conversion to vitamin A, enhancement of immune system, and protection against diseases, such as cancer by scavenging oxygen radicals, and has also used as the colour and flavor enhancements of food, anti-aging and activation of immunity. The antioxidant activity of astaxanthin has been reported to approximately 10 times stronger than that of other carotenoids tested, including zeaxanthin, leutine, canthaxanthin, and beta carotene and 100 times greater than that of alpha tocopherol (Yousry & Nagiub, 2000). Hence astaxanthin has attracted commericial interest not only as pigmentation source but also as a potent antioxidative reagent.

Different microbial groups belonging to filametous fungi, microlagae, bacteria and archeae have been found to hold potential for the production of variety of different carotenoids (Table 3).

Table 3: Microorganisms as sources of carotenoids pigments

Organisms	Name of carotenoid	Color/ appearance
	Fungi	
Blakeslea trispora	*β-carotene*	Yellow
B. trispora	*lycopene*	Pink yellow
Mucur spp	*β-carotene*	Yellow
Fusarium sporotrichoides	lycopen	Red
	Yeast	
Xanthophyllomyces dendrorhous	*Astaxanthin*	Red
Rhodotorula glutinis	*Zeaxanthin*	Pink red
	Microalgae	
Dunaliella salina	*β-carotene*	Red
Haematococcus pluvialis	*Astaxanthin*	Pink red
Chlorella zofingiensis	*Leutin*	Yellow
Chlorella zofingiensis	*Astaxanthin*	Red
	Eubacteria	
Flavobacterium sp.	Zeaxanthin	Yellow
Brevibacterium KY-4313	canthaxanthin	Orange yellow
Corynebacterium michiganense	canthaxanthin	Green- to- cream
Brevibacterium 103	Astaxanthin	Orange yellow
Bradyrhizobium sp	canthaxanthin	Dark red
	Archeae	
Haloferax volcanii	Lycopene	Cream
Halobacterium salinarium	Astaxanthin	Red
Sulfolobus shibatae	Zeaxanthin glycosides	Red
Sulfolobus solfataricus	Zeaxanthin glycosides	Dark red

Sources: Nelis & De Leenheer, 1989; Kim *et al.,* 2006; Lampia *et al.*, 1985; Lampia *et al.*, 1985; Rouka & Mantzouridou, 2001; Dufosse 2006; Yousry & Nagiub, 2000 Sardaryan 2004; Goodwin 1980; Marusichw & Bauernfeinjd, 1981).

Textile pigments from microbial sources

At present, the textile sector only use the synthetic dyes. Not only their production techniques but also their uses to manufacture colored fabrics or yarns have a tremendous environmental impact (De Santis *et al.*, 2005; Rai *et al.,* 2005; Osman *et al.*, 2004). Hence, there is a growing demand for eco-friendly/non-toxic colorants`, specifically for health sensitive applications such as coloration of food and dyeing of children textile and leather garments (Velmurugan *et al.,* 2010). Several research projects have so far been carried out to evaluate the techno-economic feasibility of today's alternative dye crops (Cerrato *et al.*, 2002; Marotti ,1997; Moresi *et al.*, 2001; Kokubun *et al.*, 1998). Among the plant species examined, common madder (*Rubia tinctorum* L),(Moresi *et al.*, 2001; Angelini *et al.,* 1997), woad (*Isatis tinctoria* L) (Kokubun *et al.*,

1998) and weld (*Reseda luteola* L) (Cerrato *et al.,* 2002) proved to be quite interesting sources of red (alizarin), indigo (indigotin) and yellow (luteolin) dyes respectively, either for their agronomic characteristics or for their dyeing properties. These three dyes were extensively exploited until the commercial success of their synthetic analogues. The main disadvantage of these natural dyes lies in the high cost of extraction and purification thus limiting their application to high-value-added natural-colored garments only (De Santis *et al.*, 2005). To tackle this constraint, it was suggested to exploit the potentiality of other biological sources such as fungi, bacteria and cell cultures, since appropriate selection, mutation or genetic engineering techniques are likely to improve significantly the pigment production yields with respect to wild organisms (Mapari *et al.,* 2005). Fungi are evaluated as potent pigment producing microorganisms Babitha *et al.,* (2007). The importance of pigments such as anthraquinone, anthraquinone carboxylic acids, pre-anthraquinones extracted from filamentous fungi. Nagia & EL-Mohamedy (2007) and De Santis *et al.,* (2005) have both reported the application of these fungal pigments in dyeing of cotton, silk and wool.

Studies showed that pigments extracted from different species of fungi including *M. purpureus*, *Emericella* spp. and *Penicillium* spp are non aromatic and biodegradable posing no toxic effects (Youssef *et al.,* 2008; Martinkova *et al.,* 1995). Fungal pigment obtained can be spray dried or lycophilized to get various colorants in powder form and solvent ethanol can be recovered by suitable solvent recovery system. The approach of reducing pollution load in the dyeing process with the use of microbial pigment at optimized conditions provides a new possibility for eco-friendly dyeing process.

Perspective and Conclusions

Microbial products production, characterization and formulation at laboratory scales, pilot scales and industrial scales are a priority applied research area as bio-resource recovery and development in both developed and developing nations. These microbial produced products include microbial pigments, alcohols, amino acids, nucleic acids, proteins, lipids, polysaccharids, microbial biomass, bio-fuels, microbial enzymes, antibiotics, bioactive compounds, flavoring agents, insecticides, growth hormones of plants and animals to mention some of them. Today none of this highly valuable products being produced by microbial strain isolated from Ethiopia, although we are buying them and using them in our daily life activities. Besides, these microorganisms continually wiped out as a result of environmental degradation, climate change and anthropogenic activities. As a result targeted isolation and preservation of microorganisms producing certain types of products should be given precedence.

One of these microbial products is pigments. Microbial pigments have various application including food coloring agents, food preservative, feed supplements, printing inks, textile dyeing, pharmaceutical and cosmetic coloring and serve as bioactive compounds. Starting from the middle of the ninth the century coloring of the above mentioned products has been done by coloring substances obtained from chemical synthesis. But at present interests in replacing or/and supplementing chemical sources of coloring substances by those obtained from biological sources are increased because of the healthy risk of the former sources of coloring substances.
Biological pigments can be obtained from plants, animals and microorganisms. The production of pigments from plants and animals sources has many limitations including: batch to batch variations, seasonal based supply and unstability under certain environmental conditions, like low or high pH and variations in temperature.
Productions of pigments, from microbial sources have many advantages, such as increasing the productivity of the producing microorganisms through fermentation process development and genetic engineering.

Pigment producing microorganisms are found everywhere and selections, isolation, characterization of pigment producing microorganisms from different environment and optimization of both nutritional and environmental conditions with elite pigment producing strains, development of culture collection centers for industrial microorganisms are the direction of future research in the microbial product development and formulation and industrialization in Ethiopia.

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