

Food additives: production of microbial pigments and their antioxidant properties

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Synthetic food additives pose the risk of hazardous effects and toxicity to the consumers whereas the application of natural pigments as food additives is safer and in demand worldwide. Food industry worldwide is dependent on colors to make food appealing to the consumers and to add variety to the food types. Therefore, a need exists to explore the novel strains of microorganisms and suitable strategies for commercial production of microbial pigments, to meet the high demand of microbial pigments as food additives. Traditional methods of microbial isolation, development and extraction are now replaced with novel techniques and strategies through biotechnology; with the advent of fermentation technology and genetic engineering techniques. Current review describes the immense potential of microbes being used as food colorants and we also present an insight on novel strains and production methods which can be explored further for application in food industry.

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Introduction

US FDA states that “any substance that is reasonably expected to become a component of food is a food additive that is subject to premarket approval by FDA”. Microorganisms and plants produce certain substances which exhibit different colors due to selective color absorption; these substances are termed as pigments. These pigments are extracted and utilized in pharmaceutical industry, textile and dyeing industry, food & dairy industry and cosmetics industry [1^{**},2,3^{**},4,5^{**},6^{**},7]. Naturally occurring pigments like isoprenoids, alkaloids and flavonoids, have been used for fragrance, flavor and color in various food types since prehistoric times [1^{**},8,9,10]. According to various studies

conducted recently it is well evident that pigments extracted from microbes are beneficial over synthetic pigments and pigments that are extracted from plants owing to their stability, availability due to no seasonal variations, cost-effectiveness, high yield through strain improvement [5^{**},6^{**},11] and smooth downstream processing for extraction [12]. Also, there exists a recent trend of public awareness on adopting environmental friendly and human safety measures [13]. Consumers have developed aversion toward application of synthetic food colorants; therefore natural food colorants are in huge demand [14,15].

In past various research studies have been conducted to explore the existence of diverse coloring pigments in plants and microbes [4,11,16]. Microbial strains producing pigments can be isolated, extracted, characterized and purified from different environmental sources like — soil, water, plants and animals [17,18]. Recent research studies indicate the immense potential of microbial pigments in the food industry and possibility of discovering novel strains from various unexplored sources like agro industrial waste, marine fungi, and filamentous fungi etc. [19,20^{*},21,22].

Recent review study states that currently there are almost 2500 types of food additives being used globally and US FDA lists around 3000 ingredients in the food additive database [23]. It also states that almost 200 thousand tones of food additive are being used per year; which highlights the fact that western diet consists of approximately 75% of the processed food. Each person’s average annual intake of food additives is estimated to be 3.6–4.5 kg [23].

Food items that are rich in nutrients, flavor, aroma and texture cannot be consumed unless the color exhibited makes its appearance relishing. Worldwide consumers are fond of colorful food products and food dishes [3^{**},24]. Microbial pigments not only add color to food, they also have populous medicinal properties like antioxidant, antimicrobial, anticancer, immunoregulation, anti-inflammatory, antiproliferative, and immunosuppressive etc. [1^{**},3^{**},25]. Most commonly used food grade pigments are β -carotene, arpink red, riboflavin, lycopene and Monascus pigments [1^{**},3^{**},12].

The production of many currently authorized natural food colorants has a number of disadvantages, including a dependence on the supply of raw materials and variations in pigment extraction. Currently, fermentative large scale

production of natural food colorants is feasible in the global market [69]. Maximization of the pigment yield while minimizing the production costs has been the attention of current techniques applied to manufacture microbial pigments at large-scale. It has also been reported that process optimization techniques have deployed statistical experimental designs and response surface analysis along with limited use of artificial intelligence like genetic algorithms [52]. Potential of renewable sources like fruits, vegetables, lichens, and marine life etc. for novel commercial food colorants is questionable with respect to raw material's availability and high investments which are recurring. Commercial production of microbial pigments for application in food industry has been attained as a result of variety of techniques combined together, namely—fermentation techniques (solid or submerged state), chemical modifications, production using agro-industrial wastes, genetic modification techniques etc. [56,71^{••},82]. Previous research studies demonstrate that commercial production of microbial pigments remains to be in the research and development stage [3^{••}].

Current literature review article elucidates the current scenario of microbial pigments as food colorants and underlines the importance of investigating large scale production strategies for microbial pigments, novel strains of microbes producing colored pigments and techniques aiding in high yield-extraction of colored pigments using microorganisms.

Why microbial pigments as food additive?

Consumers' ability to differentiate between the benefits of microbial pigments and hazardous effects of synthetic pigments has greatly boosted the application of microbial pigments as food additive. According to current trends consumers' tendency to interpret utilization of synthetic pigments as mere contaminants has been augmented [26]. Robust development and advances in technology and genetic engineering techniques have enabled food industry to produce microbial pigments [27–30]; this has led to increase in demand of natural food additives. In current times public's interest in synthetically extracted pigments has decreased owing to their toxicity, oncogenicity and teratogenic properties whereas microbial sources of pigments have gained consideration as safe alternatives [1^{••},16,23]. Precursors used in the production process of synthetic pigment have many carcinogenic hazardous effect on the workers as well as the waste produced by the production process is harmful, environment-unfriendly and non-biodegradable [3^{••}]. Worldwide interest is in the process of development for the production of pigment from natural sources [4].

Recent review states that studies conducted to observe the effects of consumption of large amount of synthetic food additives by humans leads to gastrointestinal problems,

respiratory disorders, dermatologic issues, and neurologic adverse reactions [31,32]. There exists extensive research evidence which demonstrates the association between hypersensitivity, childhood hyperactivity, physical and mental disorders with food additive intolerance [33,34]. Synthetic pigments like amaranth, erythrosine and tartrazine are potentially toxic to human lymphocytes cells *in vitro* and they have the ability to bind directly with DNA [35].

Pigments extracted from plants have drawbacks such as instability against light, heat or adverse pH, low water solubility and are often non-availability throughout the year [12,3^{••}]. The pigment production from microorganisms is efficient and beneficial process as compared to chemical synthesis of pigments [3^{••},8,36]. Likewise, extraction of pigments from microbes is not dependent on weather conditions, which gives ability to get several color shades while being cultures grown on cheap substrates [1^{••},11] and these colors are biodegradable and environment-friendly. Microbes can be grown easily and fast in the inexpensive culture medium and are independent of weather conditions. Microbial pigments possess numerous clinical properties like antioxidant, anticancer, anti-proliferative, immunosuppressive, treatment of diabetes mellitus etc. [1^{••},9,25]. Role of food colorant as additive in food industry is very important not only for the consumers but also for the manufacturers; colors and variety in the food leads to acceptability of the food product amongst the consumers which further leads to greater demand in market and hence earns large amount of profits [37,38].

Food colorants and global market

Food colorant industry market has been noted to grow at the rate of 10–15% annually. A research report published by Leatherhead Food International (LFI) (www.leatherheadfood.com) states that by 2015 the global market for food grade pigment is expected to rise by 10% to be \$1.6 billion USD [70[•]]. As per the current legislation by European Union 43 colorants are approved and permitted as food additives and approximately 30 additives are approved in the United States; 6 colorants amongst these 30 additives are of microbial origin as listed in Table 1. However, in both the regions EU and USA listed color additives are pigments extracted from natural sources mainly plants, fruits, vegetables, animal and microorganisms [39]. In addition to the food colorants there are 19 approved commercial food additives extracted from microbial source and being used globally (Table 2 lists the approved food additives) (<http://www.fda.gov>).

Microbial pigments production technologies and challenges

Algae, fungi and various types of bacteria have been used as source for commercial production of microbial pigments to be used as food additives and colorants [2,8,10,15,40,55]. Extraction of colored pigments plays pivotal role in

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