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Current perspective on bacterial pigments: emerging sustainable compounds with coloring and biological properties for the industry – an incisive evaluation

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The current inclination towards exploiting bacterial pigments for various coloring functions, like food, cloth, painting, cosmetics, pharmaceuticals, plastics *etc.* is a well-recognized aspect. Nevertheless, the current bacterial pigment productions are not effective to meet their industrial needs. Current research going on world over on bacterial pigments signify that genetic engineering for strain improvement, optimization of bioprocess modelling and utilizing cheap agro-industrial residues as substrates are key developmental strategies to maximize pigment production from bacteria. Incidentally the superior performance characteristics of the bacteria for producing differing colouring compounds and the environmental acceptability of bacterial pigments are very encouraging factors to promote higher pigment production taking advantage of the current developmental strategies. This paper evaluates the current advances in bacterial pigment production, its recovery and wide-ranging scope of its industrial applications and commercial viability.

Introduction

The quest of biotech industries for the discovery of novel bacterial pigments with superior productivity to retain their global competitiveness is persisting. Research investigations *via* purified bacterial pigments have exposed their potential for applications in food, cloth, painting, cosmetics,

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Claira Arul Aruldass pursued her degree in Bachelor of Biotechnology Industry (Hons) in Universiti Industri Selangor, Malaysia from 2006–2009. Her final year project was on the total phenolic content in Cinnamomum verum. She continued her education in M.sc of Pharmacology in Universiti Sains Malaysia from 2010–2012. Her master project is on the pharmacological studies of Mesua ferrea. Currently, she is pursuing her Ph.D in Chemistry in Universiti Teknologi Malaysia under the supervision of Prof. Dr Wan Azlina Ahmad. Her project is mainly on the mechanism of antibacterial action of violet fraction from Chromobacterium violaceum UTM5.

pharmaceuticals, plastics *etc.* As a result, bacterial pigments have become one of the key bio-products of the day with immense industrial implications which could be exploited optimistically in a sustainable environment. In addition to their potential for industrial applications, bacterial pigments also possess various functions and taxonomic significance as shown in Fig. 1.

Over other pigment producing microbes, bacteria offers certain distinctive advantages, owing to their short life cycle, compatibility to season and climate, potent to produce pigments of different colors and shades, easier to scale-up production of pigments *etc.* Some examples of the pigment producing bacterial species include *Flavobacterium* sp. (yellow pigment, zeaxanthin), *Agrobacterium auranticum* (pink-red pigment, astaxanthin), *Micrococcus* sp. (various colored pigments, carotenoids), *Pseudomonas aeruginosa* (blue-green pigment, pyocyanin), *Serratia marcescens* (red pigment, prodigiosin), *Chromobacterium* sp. (violet pigment, violacein) and *Rheinheimera* sp. (blue pigment, glaukothalin).[1] Biotechnology could be a solution for providing additional pigments including interesting aryl carotenoids, isorenieratene (Φ, Φ-carotene) and its monohydroxy and dihydroxy derivates can be produced by bacteria *i.e. Brevibacterium linens*, *Streptomyces mediolani* and *Mycobacterium aurum*.[2]

The success of any microbial pigment product manufactured depends upon its acceptability in market, regulatory approval and the size of capital investment required to bring the product to market.[3] The successful marketing of food grade microbial pigments like riboflavin, β-carotene and phycocyanin reflects the importance of niche markets in which consumers are willing to pay attention for natural pigments. Carotenoids market has resulted interesting in 2010 estimated at nearly $1.2 billion, but the expectations for 2018 are increasing considerably supposing to reach $1.4 billion with a compound annual growth rate of 2.3.[4]

Successful commercialization ventures for bacterial pigments are normally hampered by the high cost of synthetic growth medium. Efforts were made to reduce the production cost of pigments and in this view, various studies have been carried out to explore the possibility of using other types of cheaper growth medium and agricultural waste to perform this type of bioprocesses which can also reduce its environmental impact.[1] Recent developments in the molecular biology have provided a variety of genes that can be employed as tools for a new strategy of heterologous expression in different host organisms. Engineering of microbial pathway enzyme can also produce high amount of pigments in industrial process.[5]

Regardless of these factors, further efforts are vital for higher production of bacterial pigments for industrial and commercial exploitation, which remain untapped. This review aims to disseminate the current knowledge on higher production of bacterial pigments by providing a summary of the present understanding and recent developments in the production of bacterial pigments and in that way draws the attention of researchers and stakeholders to explore and exploit this over flowing resource economically.

Professor Laurent Dufossé has a position at Reunion Island University, located in the Indian Ocean, nearby Madagascar. Previously, he was researcher and senior lecturer at the Université de Bretagne Occidentale, Quimper, France. He has been involved in the field of Biotechnology of Food Ingredients for 25 years. Before joining the University he was Research Project Leader at private companies such as Sanofi-SKW or Lesaffre. His main research subject is focused on microbial production of pigments. This activity started 16 years ago and studies were and are mainly devoted to aryl carotenoids, such as isorenieratene, C50 carotenoids and anthraquinones.

Dr Zainul Akmar Zakaria is a Senior Lecturer at the Institute of Bioproduct Development, Universiti Teknologi Malaysia. Has been with the university after completing his secondary education at Victoria Institution, Kuala Lumpur in 1993. He is a chemist-turned environmental biotechnologist with specialization in the field of metal–bacteria interaction. To date, he has 19 Scopus-indexed publication to his name, with citation of 140 and h-index – 6.

Prof Dr Wan Azlina Ahmad has been involved in the area of natural pigments for more than 15 years. Starting from the first report on the presence of anthocyanins in oil palm fruits in 1985, her work has progressed to the pilot scale production of bacterial pigments, notably prodigiosin and violacein. The many uses of the bacterial pigments especially in the area of textile dyeing, food applications and as antibacterial agents are being looked into. Her other interests include metal–microorganisms interaction and how this can be exploited for the benefit of mankind.

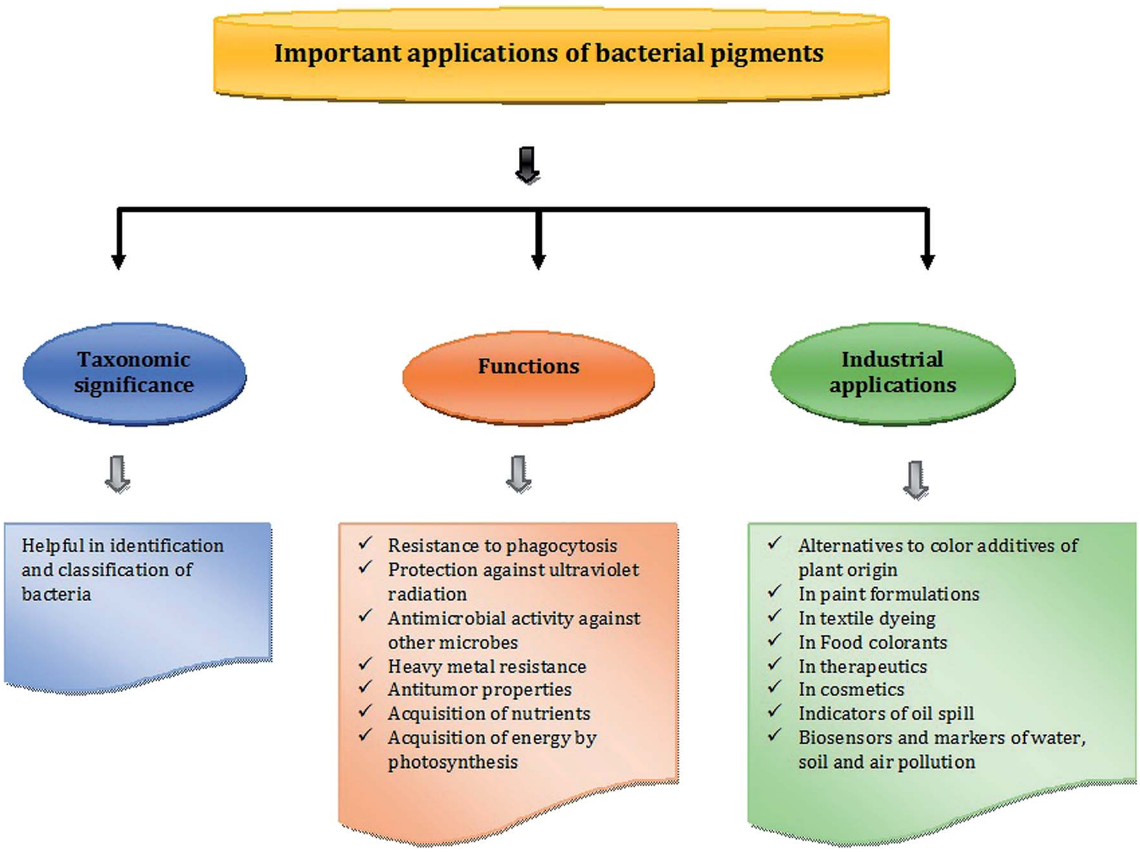

Fig. 1 Aspects of bacterial pigments.

Bacterial pigments

Bacteria have been exploited commercially for the production of pigments.[6–8] The nontoxic nature together with easy separation from cell biomass are the added advantages of bacterial pigments. The production can be increased in geometric proportions through genetic engineering compared to scaling up and further research can overcome the quantum leaps in the economics of bacterial pigments.[9] Pigments can be classified based on their structural affinities. Some of the most important bacterial pigments, their structures and applications are given in Fig. 2.

Genetic engineering for strain improvement

The motivation for industrial strain development is economic, since pigment concentrations produced by wild strains are too low for economic process. It is very important to isolate strains which produce pigments with shorter fermentation times.[10] With the integrated knowledge of biochemistry, chemical engineering and physiology, microbiologists have taken a more scientific approach to the identification of bacterial strains with desired traits. The wild strains cannot make the product of commercial interest at high yields to be economically viable. To improve the bacterial strains, the sequence of genes in the DNA must be altered and manipulated and these alterations will enable the bacteria to devote their metabolic machinery to produce the key compounds and increasing the product yields. The strain improvement by ultraviolet (UV), ethyl methane sulfonate (EMS) and 1-methyl-3-nitro-1-nitrosoguanidine (NTG) is convenient and can yield a several fold enhancement of pigments, as proved in few cases.[11] Liu *et al.*[12] have used microwave as a mutant agent to mutate *Serratia marcescens* jxl-1 with high yield and high purity prodigiosin pigment which has promising prospects in food, cosmetics and textile industry. We can expect other biotechnological applications of bacterial prodigiosin in the near future.[13]

To understand the promising nature of the pigment production, whole genome sequencing of the bacteria has to be carried out. The draft genome data were analyzed by Puranik *et al.*[14] for biosynthesis of pigment from *Pseudogulbenkiania ferrooxidans* strain and the strain showed homology with the reported pathway from *Chromobacterium violaceum*. Violacein has shown potential economic importance for industrial purposes offering the unique applications in cosmetics, textiles as well as in agro-industry.[15] The biosynthesis pathway will show the presence of various important categories like secondary metabolism, stress response and iron acquisition metabolism and aromatic compound metabolism. These categories involve in distant cause relationship with the violacein production being under the influence of a quorum-sensing mechanism.

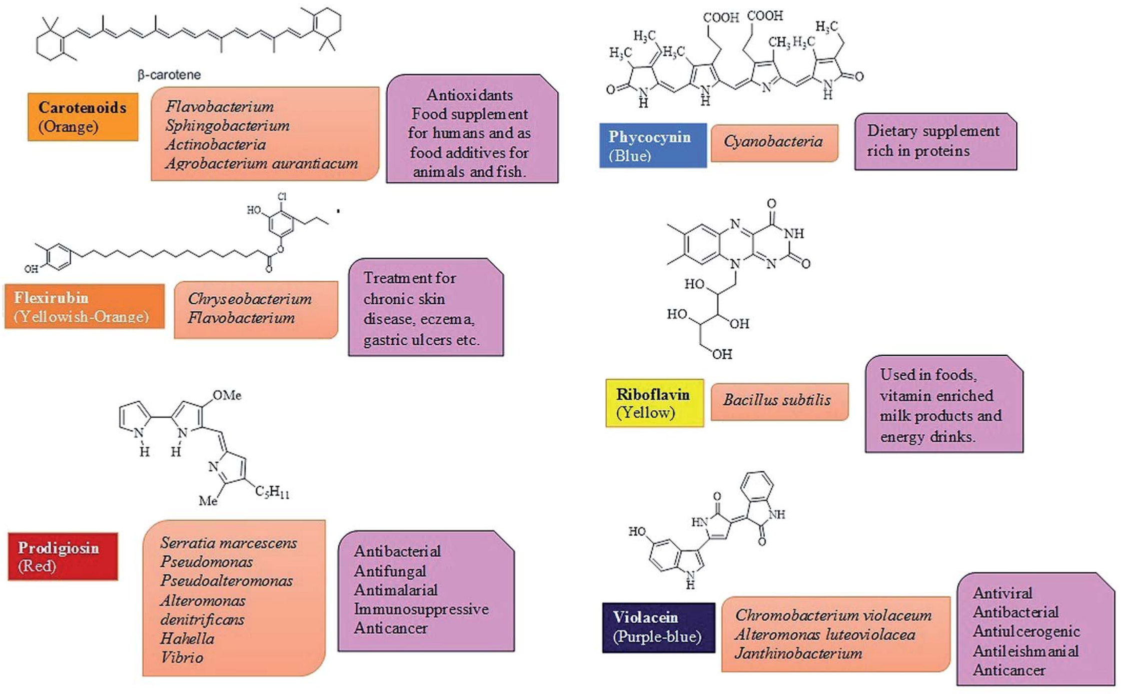

Fig. 2 Structure of bacterial pigments.

Identification of such genes and investigations of the promotor regulation through sequence analysis using B-PROM could reveal strategic interrelations of networking, which might elucidate the molecular aspects of pigment production[16,17] could be used for rapid screening of pigment-producing bacteria from unique habitats with potential secondary metabolites of commercial value which might open new avenues for industrial production of bacterial pigments.

Recent developments for enhancing bacterial pigment production

Bacterial pigments can be produced biotechnologically but they synthesize relatively low concentrations of pigments and cannot compete economically with synthetic pigments.[18] To meet the growing demands in the pigment industry it is necessary to improve the performance of the bacteria and thus increase the yield. The biocolorants identified by their chemical name can be synthesized easily by cheaper biotechnological sources.[19] The growth and pigment production of the bacteria are strongly influenced by medium composition and can be improved by supplementation of the culture broth by stimulators and the optimization of culture conditions *via* statistical experimental designs – response surface methodology (RSM), Artificial Neural Networks (ANN) *etc.*

Plackett–Burman design was applied in the first step of optimization to determine the likely effects of medium components on pigment production. The factors having the significant effects were optimized in the second step using a central composite design and the response of each variable was recognized by the regression analysis. Based on the regression analysis, media were optimized for the large scale production.[20] Artificial neural network (ANN) is a powerful modeling technique that offers several advantages over other modeling techniques and have been applied to simulate processes such as fermentation. Nowadays, RSM combined with ANN approach is applied for optimization and process modeling for enhancing pigment production.

Immobilization cultures are also currently used to enhance cell proliferation and the production of bio products. Yamashita *et al.*[21] found that the addition of porous silica gel and aerosols immobilized bacteria considerably increased the production of prodigiosin. Therefore, supplementing solid carrier may be effective as increasing prodigiosin production. According to Chen *et al.*[22] the addition of alginate beads as a porous carrier further increased the production of prodigiosin from 7.05 g L^{-1} to 15.6 g L^{-1}. Also, there is an increase in pigment production when the cells are exposed to stress conditions which may include elevated temperatures, osmotic pressures, metabolic inhibition, and existence of heavy metals and so on. If bacteria are able to adapt to these stress conditions then their growth is restrained and the yield of pigment from fermentation is increased.

Pigment production on an industrial scale is not economical since the cost of technology used is still high and researches have led to find out low-cost substrate as alternative to reduce production cost. Therefore in recent years, various agro-industrial residues have been used as a substrate or additive for pigment production which may represent an added value to the industry and meets the increasing consciousness for energy conservation.[4] Application of agro-industrial residues in bioprocesses provides alternative substrates and also helps in solving pollution problems which their disposal may otherwise cause[23,24] corn meal,[25,26] peanut meal, coconut residue, soybean meal, jackfruit seed, tapioca starch,[27] grape juice, grape must,[28] peet extract,[29,30] mustard waste, mung bean waste, sugar beet molasses and corn syrup[31–33] have been used as substrates as source of carbon, starch, vitamins and minerals for the production of pigments. There may be many other factors affecting pigmentation by the bacteria and a thorough understanding of the effect of these factors and regulation of biosynthetic pathways for pigment production will help to develop a controlled bioprocess for the enhanced production of desired pigment, thus opening the new avenues for further research in this field.[34]

Processing techniques

Bacteria offer a tremendous source of various pigmented molecules, yet separation and purification of pigments at industry level is a bit tedious. Numerous methods have been proposed to improve both pigment extraction and purification efficiency.[35,36] But many of them have the drawback of high temperature requirement, long processing time and extraction using organic solvents which can result in the degradation and formation of unwanted isomeric components.[37,38] Enzymatic treatment has also been proposed as an alternative stage and had practical limitations, due to the use of expensive commercial enzyme.[39] Several research projects on separation and purification has been investigated all over the world and it seems that new technology is required for effective separation process.

Wang *et al.*[40] used non-ionic adsorption resins for the separation and purification of the pigments. In this method, the targeted pigment could be adsorbed on the selected resin directly from the culture broth and this technology can lower the cost of operation because of its low consumption of solvents and reusable adsorbents. Besides eliminating cell separation step, this method yielded a concentrated and partially purified product with total recovery of 83%, which was much higher compared to other conventional methods. Therefore, this method is interesting to be applied in industries to reach the maximum possible purity. Apart from this, several technological advances are still necessary to improve the separation and purification of pigments from culture broth to reduce the energy and cost intensive process.

Some bacterial pigments are sensitive to light, heat, pH and insolubility in water and therefore have poor stability and easily degradable, which is the most important concern to be studied. Certain modifications will help increasing the solubility and improve the stability of the pigments.[41] Genetic engineering of the pigmented bacteria will help improving the pigmentation process and further intense research is required to study the stability of the pigments.

Industrial production of bacterial pigments (lab scale – pilot scale)

Nowadays, the importance of biotechnological processes has increased due to benefits that they provide such as high yields, low costs and less waste disposals. To design a biotechnological process, many factors such as bioreactor design, raw materials used, bacteria, pigment, type of fermentation (batch, feed-batch or continuous) must be taken into consideration. These features play a very important role to achieve the desired yields of a target pigment.[42] The configuration and volume of the

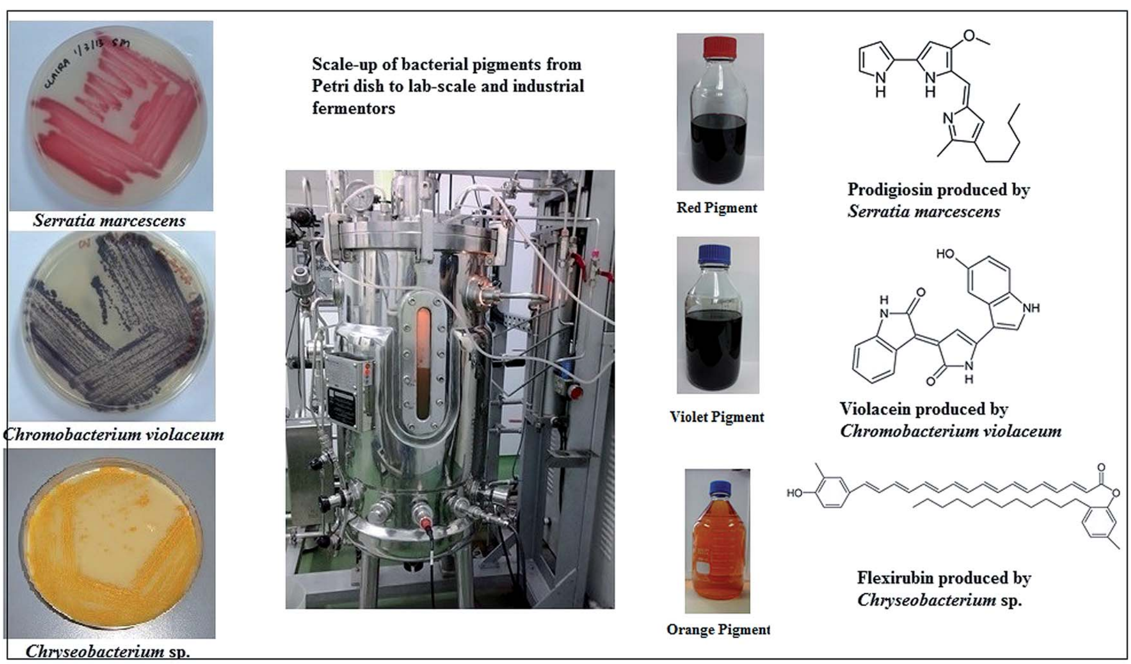

Fig. 3 Pilot scale production of bacterial pigments.

bioreactor is an important factor to consider in the production of bacterial pigments. A fully functional bioreactor offers advantages such as perfect integration of several components, ensuring that cultures will reach the desired productivity of microbial pigments or other microbial compounds, through an efficient and rigorous control of some parameters such as temperature, agitation, aeration, pH and dissolved oxygen among others. Industrial production of bacterial pigments generally employ genetically modified strains to maximize pigment production and metabolic engineering being an interesting field, triggers pigmentation. Fig. 3 shows the scale-up of bacterial pigment production from lab scale to industrial fermenters.

Recently, Aruldass *et al.*[43] reported that prodigiosin is produced by *S. marcescens* UTM1 using brown sugar as a low cost medium in a 5 L stirred-tank bioreactor. The growth of pigmented bacteria is a bioprocess, which is regulated by a complex interaction between the physical, chemical and biological conditions of the living environment of fermentation and the biochemical process inside the cells. Physical factors such as temperature, pressure, agitation rate, power input, flow rates and mass quantities are the most important parameters during fermentation in a bioreactor.[44] For instance: *S. marcescens* was cultured in the bioreactor, most of the oxygen is transferred in the region near the impeller. Oxygen diffuses from the culture medium, through the wall of tubing and into a flow of inert gas passing through the tube. Thus, more oxygen is consumed by *S. marcescens* during fermentation and the oxygen concentration gradually decreased. Oxygen consumption promotes the growth of the bacteria and simultaneously initiate the prodigisoin production. This metabolite was synthesized by a bifurcated pathway during the enzymic condensation of the terminal products of the two pathways, 2-methyl-3-*n*-amyl-pyrrole (MAP) and 4-methoxy-2-2-bipyrrole-5-carbaldehyde (MBC) precursors.[45] The proteins responsible for biosynthesis of MAP and condensation with MBC to form prodigiosin were identified as *pig*B–*pig*E genes in *Serratia* strains.[45,46] At the final stage, MAP and 4-hydroxy-2,2′-bipyrrole-5-carbaldehyde (HBC) are both incorporated by the condensing enzyme, *pig*C to form prodigiosin.

The fermentation process is normally carried out at a constant pH. However, the pH of the culture medium gradually decreases with the prodigiosin production from *S. marcescens*. The pH is an important factor in *S. marcescens* fermentation because it influences the breakdown of substrates and transport of medium substrates and pigments through the cell wall. Small change in the pH may cause a great fall in the pigment productivity. Fermentation of *S. marcescens* may cause formation of froth due to rapid rise and dispersion of bubbles as gas introduced into the culture medium. Song *et al.*[47] used a novel integrated bioreactor with an internal adsorbent (HP-20 resin) to increase the production yield and allow easy recovery of prodigiosin. It was reported that the adsorption of prodigiosin is dependent on pH and is increased in the neutral pH range and decreased in the alkaline-pH range. In the downstream processing, the prodigiosin was extracted from culture by using organic solvents such as ethyl acetate or acetone. Thus, an efficient operation downstream processing operations are vital elements in isolating the pigments.[48]

Mohanasrinivasan *et al.*[49] fermented *Streptomyces coelicolor* $MSIS_1$ to obtain amaranth colour pigment in a 5 L bioreactor using mannitol and soybean flour supplemented GAUSE'S Medium and amaranth pigment was successfully used as pH indicator in citrate utilization test. Carotenoids, particularly canthaxanthin was extracted from *Dietzia natronolimnaea* HS-1 using continuous culture strategy in 2.5 L bioreactor with supplementation of enzymatic hydrolysate molasses as an ideal carbon source. This pigment was suggested to use as natural antioxidant and food preservatives.[50] Naik *et al.*[51] used an agro-based substrate, peanut oil cake as low cost medium for the production of prodigiosin from *Serratia marcescens* CF-53 in 2 L bioreactor. Despite traditional bioreactors, stirred-tank and air lift reactor, trickle-bed reactor is an emerging technology in fermentation aspects. Pigment may continuously be collected as effluent from the bioreactor as the solution of substrate pass through enzyme immobilized in tube or bed. This may recover the pigment at lower separation cost than other homogeneous systems. Thus, designing a suitable culture system with suitable bioreactor for industrial fermentation is necessary to attain high pigment production.

Commercialization/market potential

Successful development of bacterial pigments coupled with economic feasibility of using agro-industrial residues minimize the cost for commercial production and marketing. Enabling successful advancement and commercialization of bacterial pigments will require the confidence and engagement of key public and private stakeholders so that they can make necessary investments to reduce the technical risks and overcome the challenges associated with developing bacterial pigments in industry.[52] The bacterial pigments will offer good opportunities due to their enhanced environmental acceptability and superior performance characteristics and are expected to continue to dominate the organic market.[10]

Conclusions

Bacterial pigments have economic potential and industrial importance offering opportunities for applications in textile, food, pharmaceuticals, cosmetics *etc.* But their current volume of production still has not attained the optimum level to meet the demand aroused due to the recent awareness for natural products. The current novel strategies like genetic engineering, molecular biology techniques and fermentation technologies are greatly contributing to higher production of bacterial pigments. For cost-competitive and higher production of bacterial pigments, these current processes of screening of new pigmented bacteria should continue in order to support the discovery and application of novel bacterial pigments that possesses high activities and useful properties from less expensive sources.

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