Review Paper:

Violacein: A Promising Bacterial Secondary Metabolite

Kanade Yogini[1], Mohan Waman[2] and Patwardhan Rajashree[1*]
1. Department of Microbiology, Haribhai V. Desai College of Arts, Commerce and Science, 596, Budhwar Peth, Pune 411002, Maharashtra, INDIA
2. Dr. D.Y. Patil Arts, Commerce and Science College, Akurdi, Nigdi Pradhikaran, Pune, Maharashtra, INDIA
*dr.rbpatwardhan@gmail.com

Abstract

Pigment is a secondary metabolite produced by microorganisms. As a natural product, bacterial pigments are being studied for their applications in pharmaceutical, food and cosmetic industry. Purple and blue bacterial pigments are less common. Violacein, a purple pigment is an indole derivative with molecular mass of 343.3. Some bacteria like Pseudoalteromonas sp. 520P1, Chromobacterium violaceum, Janthinobacterium lividum, Duganella violaceinigra, Collimonas and Iodobacter species are reported for violacein production. It is produced by vio operon comprising of vioABCDE genes. Violacein production is beneficial to the bacterial cell as it acts as a respiratory pigment involved in the regulation of tryptophan production, active in defensive mechanism and helps cell survival in stressed conditions. Carbon source, pH, temperature, incubation period and agitation rate are factors affecting violacein production. Violacein has shown antibacterial, antifungal, antiviral, anticancer and antiparasitic activity. It also displayed immunostimulatory, gastroprotective and antioxidant properties. Violacein gained industrial importance as a coloring agent in cosmetic, food and textile industries. This review intended to describe the violacein production by bacteria, its advantages to bacteria producing it, factors affecting its production and its applications.

Keywords: Violacein, pigment, indole, anticancer, *Chromobacterium violaceum*.

Introduction

Colors are part of human life. Dyeing was known to the mankind since Indus Valley period (2500 BC). Findings of colored garments of cloth and traces of madder dye in the remnants of the Indus Valley Civilization at Mohenjodaro and Harappa (3500 BC) show evidence of the knowledge of dyeing[71,74].

Natural dyes are used in the coloration of textile, food, cosmetics, paper, soaps, candle, leather, wood etc[28]. In the ancient time, man used dyes for the coloration of feathers, cave paintings and to paint faces on ceremonies[71]. Initially there were only natural sources for obtaining these dyes like plants, minerals and animals [28]. After the accidental synthesis and commercialization of mauveine by William Henry Perkin in Germany in 1856, the coal tar dyes started substituting the natural dyes[71]. Limitations of natural dyes that lead to the decrease in the demand of these dyes include:

- Availability
- Stability
- Color yield[56]

Although the synthetic dyes were used for their advantages such as chemical stability, low production cost and different color shades, they have many drawbacks like:

- Environmental pollution – leading to the harmful effects on humans and aquatic life. Xenobiotic nature of the synthetic dyes alters physicochemical properties of soil[46,68].
- Health hazards – hyperactivity in children, allergic, irritant, carcinogenic nature and respiratory problems[46].
- Water requirement - large amount of water is required especially in the textile industry for coloring the fabric[68].
- Affect agriculture productivity – toxic nature of the synthetic dyes affects the natural flora of soil leading to the death of the inhabitant microbes which in turn affects the agricultural productivity [46].

All these limitations of synthetic dyes make the recent shift to the use of natural colors. Natural colors could be obtained from pigments of plant or microbial origin[46]. Several benefits are offered by microbial pigments making microbial pigment a subject of intense research for exploring its potential for various applications[28].

There are different types of bacteria inhabiting different habitats. Some of them are known as chromo bacteria because of their ability to produce pigments of different colors. A number of different kinds of pigments are produced by microorganisms. (Table 1). Chemically bacterial pigments are Carotenoids, Xanthophylls, Pyrrole, Phenazine, Quinine or Quinone derivatives, Tri-pyrrylmethene, Bisindole[12,22].

These pigments have various applications. Their easy extraction and biodegradability enable them to be used as an alternative to synthetic dyes in textile industry[25,80]. Non-toxic and ecofriendly properties of the pigments make their use in food industry as a colorant as well in cosmetics[25,53]. Safe use of bacterial pigments as a natural food colorant can be assured from the fact that pigment producing bacteria do not have any effect on human micro flora[47]. Few bacteria produce pigments like β carotene (*Flavobacterium*, *Agrobacterium aurantiacum*), riboflavin (*Bacillus Subtilis*) which are used in food to add the nutritive value[25,53].

Table 1
Pigment Producing Bacteria

Bacteria	Pigment	Color
Staphylococcus aureus[59]	Staphyloxanthin, Zeaxanthin	Golden Yellow
Serratia marcescens[47]	Prodigiosin	Red
Streptomyces coelicolor[28]	Actinorhodin, Prodigiosin	Red
Chromobacterium violaceum[47]	Violacein	Purple
Pseudomonas aeruginosa	Pyocyanin	Blue Green
Paracoccus carotinifaciens	Astaxanthin	Pink red
Corynebacterium insidiosum[59]	Indigoidine	Blue
Haloferax alexandrinus[28]	Canthaxanthin	Dark Red
Janthinobacterium lividum	Violacein	Purple
Chryseobacterium[27]	Flexirubin	Orange
Flavobacterium specie[23]	Flexirubin	Yellow
Pseudoalteromonas sp. 520P1	Violacein	Purple

Furthermore, because of their antifungal, antibacterial, antioxidant, antitumor properties, they attract attention of pharmaceutical industry[25,45,66]. Reports showed that pigment offers benefits to bacterial cell such as heavy metal resistance. This ability can be further used for remediation of soil and water polluted with heavy metals like arsenic, cadmium and mercury [29].

Bacterial pigments offer many advantages over the synthetic dyes. These include:

- Simple and fast techniques for culturing – Simple and easy techniques are required for isolation and culturing of pigment producing bacteria.
- Simple pigment extraction techniques – Simple techniques like liquid-liquid extraction techniques are required for extraction of bacterial pigment.
- Broad ranging activities – Some bacterial pigments have shown good activities against gram positive, gram negative bacteria, mycobacteria and fungi as well. Apart from antibacterial or antifungal activities, bacterial pigments hold promise as antioxidant, anticancer and antidiabetic agents.
- Season independent – Bacterial pigment production is independent of season and there is no disparity in the color shade according to the season.
- Cost Effective – Availability and utilization of cheap substrates make bacterial pigment production on a large scale a cost-effective process.
- Safe and environment friendly – Bacterial pigment being natural source of colorant is safe to use as a food colorant and does not create any harmful effects on environment as well[47].

Purple and blue bacterial pigments are less common [20]. Few bacteria are reported to produce violacein. *Pseudoalteromonas* sp. 520P1, a gram-negative marine bacterium was described to produce violacein only under static culture conditions[32]. *Chromobacterium violaceum* [47] and *Janthinobacterium lividum*[58] are known to produce pigment violacein. *Collimonas* sp. was reported to produce violacein[43]. A newly isolated phenotypic variant of *Duganella, Duganella violaceinigra* str. NI28 was reported for its high-level production of crude violacein[29].

Genome sequence of violacein producing *Iodobacter* sp. has been drafted recently[13,20,38]. Apart from its antimicrobial activity, violacein also exhibits properties like immunostimulatory and anticancer agent[40]. Rare and multipurpose nature of violacein make it the focus area of this review. This review is an effort to compile most of the data on this versatile bacterial secondary metabolite.

Characteristics of violacein

Violacein (3-[1,2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ylidene]-1,3-dihydro-2H-indol-2-one) is a secondary metabolite reported to be produced by *Chromobacterium violaceum*, *Janthinobacterium lividum*[47,52,58,93], *Collimonas, Duganella, Microbulbifer* sp.and *Pseudoalteromonas*[30]. Its molecular mass is 343.3. It is insoluble in water, slightly soluble in ethanol, moderately soluble in dioxane and acetone and soluble in DMSO, methanol and ethyl acetate. Its melting point is >290^{o}C. The UV-Vis spectrum exhibits maximum absorbances at 258, 372 and 575 nm in ethanol[41]. It has a dimeric structure composed of 5-hydroxyindole, oxindole and 2-pyyrolidone subunits[10,73].

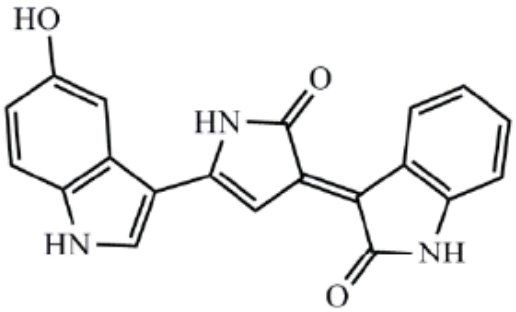
Violacein gene expression

Production of violacein involves expression of *vio* operon. *Vio* operon consists of five enzyme coding genes *vioA, vioB, vioC, vioD* and *vioE*, transcribed in one direction. A 7.3 kb long DNA fragment codes these enzymes [52]. Analysis of the violacein gene cluster sequence revealed an insert of 10,094 bp encoding five open reading frames (ORFs), *orf1* (1629 bp), *vioA* (1257 bp), *vioB* (2997 bp), *vioC* (1290 bp), *vioD* (1122 bp) and *orf2* (partial). The product of *orf1*was found to be weakly similar to phospholipase C precursor protein. Profile scan analysis revealed a conserved N-terminal NAD(P)H binding domain in each of these proteins. (Figure 3). Result of the PSI-BLAST analysis suggested that the products of the genes *vioA, vioC* and *vioD*, proteins VioA,

VioC and VioD respectively are related to the PheA (Phenyl hydroxylase)/TfdB (2,4 dichlorophenol hydroxylase) family of FAD monooxygenases. This family of monoamine oxidases catalyses the oxidative deamination of hydrophobic and aromatic L-amino acids. HPLC analysis of the structure of the intermediates produced by *vio* mutants suggests the role of *vio* gene products in the biosynthesis of violacein as described in the table 2[10]. VioB, VioC and VioD are more important than VioA and VioE in the violacein production[52].

Table 2
***Vio* operon genes and their function in the biosynthesis of violacein[5,10,40,53].**

Gene	Gene Product	Enzyme codes for	Role in the violacein biosynthesis	Molecular Weight
*vio*A	VioA	Tryptophan 2-monooxygenase	Generation of an indole pyruvate intermediate	48
*vio*B	VioB	Considered to be a polyketide synthase	Catalyzes the 1,2-indole shift of tryptophan as well as the condensation reaction to generate the violacein pyrrole ring	111
*vio*C	VioC	FAD dependent monooxygenase	Acts on prodeoxyviolacein and hydroxylates the indole ring to generate the violacein oxindole subunit	48
*vio*D	VioD	Flavin dependent monooxygenase	Additional oxygen atom incorporation on violacein oxindole subunit	42
*vio*E	VioE	Conversion of flavanone to isoflavone	Conversion of short-lived dimer indole-3-pyruvic acid imine (IPA) into protodeoxy-violaceinic acid (PVA)	22



Violacein **(104)**
$C_{20}H_{13}N_3O_3$

Figure 1: Chemical Structure of Violacein[22,23].

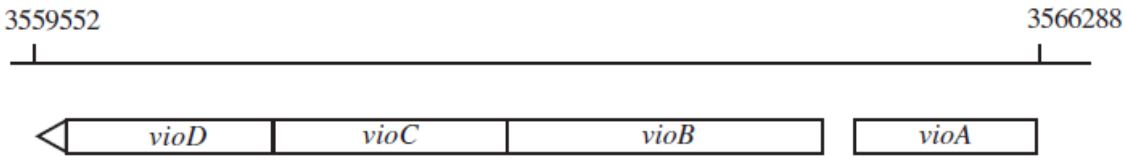


Figure 2: Schematic drawing of structural genes of the violacein biosynthesis operon[5].

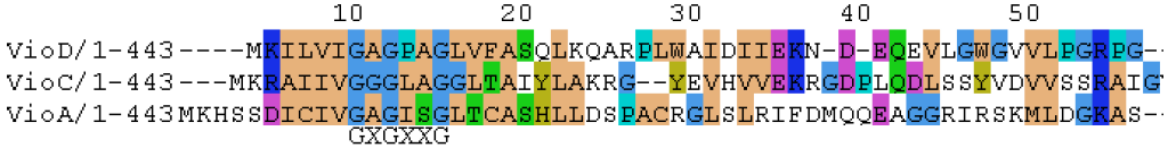


Figure 3: Alignment of the VioA, VioB and VioC proteins revealing a conserved GXGXXG nucleotide binding motif[10].

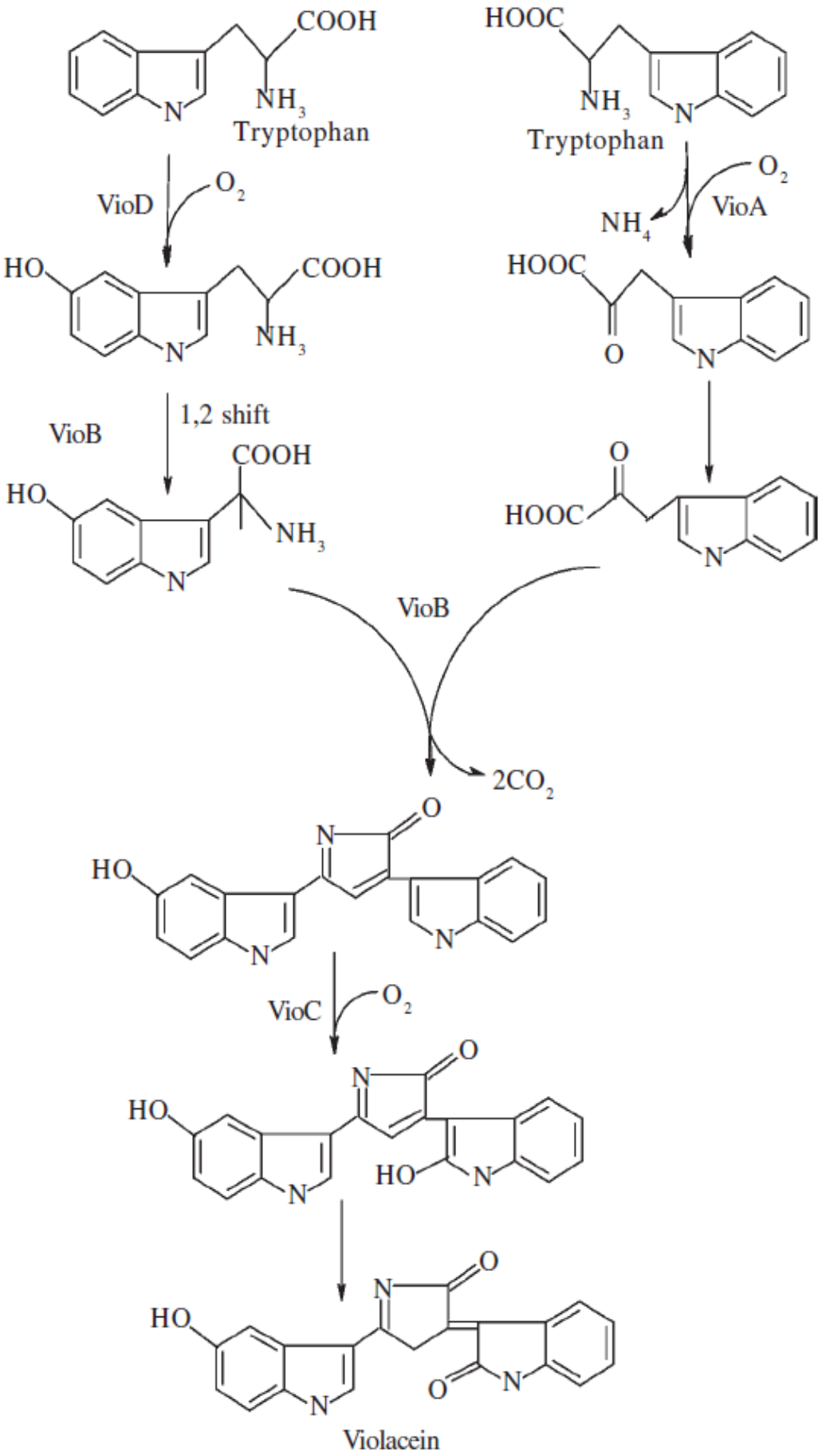


Figure 4: Violacein biosynthesis in *Chromobacterium violaceum*[5,10].

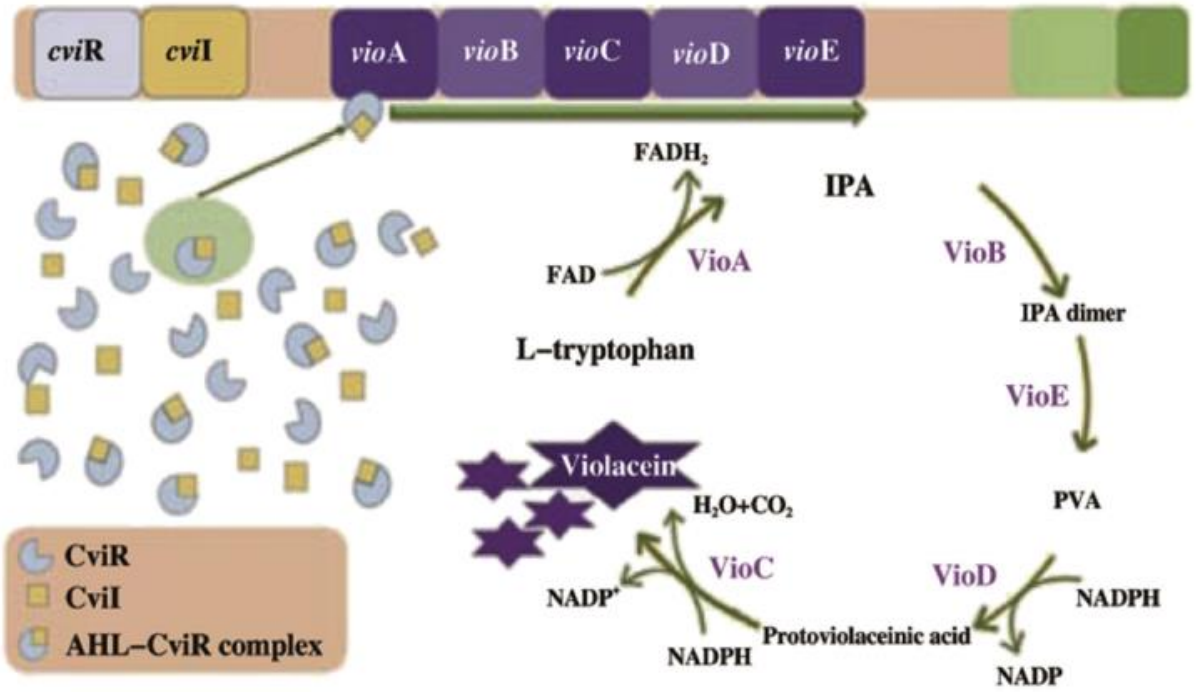


Figure 5: Schematic of violacein biosynthesis[53].

Biosynthesis of Violacein

Tryptophan appears to be the precursor molecule for the synthesis of violacein in *C. violaceum*[5]. Biosynthesis of violacein involves enzymatic oxidation of two L-tryptophan molecules. The first enzyme flavin dependent tryptophan 2-monooxygenage, product of *vio*A gene, catalyses oxidation of tryptophan into indole -3-pyruvic acid imine (IPA) along with FAD cofactor reduction. The product of *vio*B gene converts IPA into short-lived imine dimer through dimerization process. This short-lived dimer has two fates, either it spontaneously transformed into chromopyrrolic acid involved in indolocarbazole biosynthesis or the product of *vio*E gene modifies the dimer speedily into protodeoxy-violaceinic acid (PVA) which leads to the biosynthesis of violacein.

The product of gene *vio*D catalyses hydroxylation at the fifth position of one indole ring of PVA to yield proviolacein with oxidation of NADPH to NADP. Further the product of gene *vio*C converts proviolacein into violacein with the oxidation of NADPH into $NADP^+$ and formation of water and carbon dioxide[52].

A diffusible signal molecule named N-acyl-homoserine-lactone (AHL) is known to control the biosynthesis of violacein. In *C. violaceum*, an AHL identified as N-hexanoyl-homoserine lactone (HHL) has been found. An evolutionarily conserved transcriptional regulator including two adjacent genes *cvi*I encoding an auto inducer (HHL) synthase and *cvi*R encoding a receptor (regulator protein) as a part of quorum sensing system of *C.violaceum* was reported to regulate the production of violacein[5]. When growth of the *C.violaceum* reaches near the stationary phase, large amount of constitutive CviI (product of gene *cviI*) accumulates HHL. A stable protein-ligand complex forms on binding of HHL to *cvi*R operator leading to its expression. Increased amount of CviR (product of gene *cvi*R) allows it to bind to promoter site of violacein operon thereby increasing the pigment production[52].

In a strain CV026 of C. *violaceum*, violacein production is inducible by AHL with N-acyl side chains of C4-C8 length. On contrary, N-acyl side chains from C10 to C14 inhibit violacein production[63]. Similar result was reported for the *Pseudoalteromonas ulvae* TC14 where C6-, C12-, 3-oxo-C8 and 3-oxo-C12-HSLs (homoserine lactones) upregulated violacein production while 3-oxo-C6-HSL downregulated pigment production[11].

Benefits of pigment production to bacterial cell

In general, the purpose of pigment production in bacterial cell is not well characterized. In bacteria such as *Pseudomonas aeruginosa*, pigment may help the bacterium for iron uptake and plays important role in its virulence[66]. Pigments are reported to protect from UV rays and radiations which help bacteria in survival by releasing the oxidative stress that may result from harmful radiations[23]. Antimicrobial properties of pigment give a competitive advantage to the bacteria producing it[29].

Function has been ascribed to violacein from *C. violaceum*. It has been suggested that violacein production is involved in the regulation of tryptophan production [5,66]. Increased respiratory activity after addition of the violacein extracts to non-pigmented *C. violaceum* cells proposes violacein as a respiratory pigment [5]. *Chromobacterium violaceum* growth on complex and complete medium stops pigment production, indicating the pigment is not required for the

growth and survival of the organism[5]. *Janthinobacterium lividum* and *Chromobacterium violaceum* use violacein for survival as a defensive mechanism by inducing cell death of common bacterivorous nanoflagellates[23].

Stress protective activity of violacein can be evident from the observations like production of violacein in presence of sub-inhibitory concentrations of ampicillin and recovery of high number of bacteria (*J.lividum*) from death phase of growth curve from violacein producing cultures. Further, it suggests that the synthesis of violacein could take place in stressed conditions thereby resulting into the prolonged survival of cells synthesising it[67].

Factors affecting violacein production

Carbon source: Carbon source is one of the important factors affecting the biomass and the violacein production. Nutrient broth was found to be a suitable medium for violacein production followed by nutrient broth with glycerol. This could be attributed to the peptone content of NB which affects the violacein production. Glucose acts to increase the cellular biomass but inhibits the violacein production[49,93]. The inhibitory effects of glucose could be because of inhibition of even distribution of dissolved oxygen thereby creating anoxia which retard the violacein production[93]. However, in *Massilia* sp. BS-1, there was no inhibitory effect of glucose on violacein production[1]. Slow consumption carbon sources like sucrose, lactose result in an increase in the secondary metabolite production[93].

Media supplemented with tryptophan or having high amino acid content was reported to increase the violacein production. This could be attributed to the fact that tryptophan acts as the only precursor molecule for the violacein synthesis [31,35]. Addition of L-Histidine was reported to be essential for the violacein production by *Massilia* sp. BS-1 [1,35]. *C. violaceum* was reported to utilize cellulose as a carbon source and convert the sugars into amino acids thereby producing violacein[93].

Effect of pH: pH of the medium influences violacein production by different organisms. 3.8-fold increase in the violacein production was observed at pH 7 as compared with the production of pigment at pH values of 6 and 8. No pigment was obtained at pH 5 and for *J. lividum*, growth at pH 9 found to be an antagonistic[49]. Maximum rate of violacein synthesis was observed between pH 7 and 8[35]. pH also has the effect on the stability of the violacein pigment. Violacein intensity was least affected between pH 5-9. At pH 13 violacein turned to green and at acidic pH (pH 2), the pigment changed to darker blue.

At pH 11, violacein turned colourless. This is because change in pH results in the decomposition of the violet pigment. Excess of OH^- ions in alkaline conditions deprotonates hydroxyindol phenolic group and oxoindol, pyrrolidone amine group leading to the formation of anion and demolition in the conjugated structure of the pigment[93].

Effect of Temperature: Temperature has an impact on the amount of pigment produced. Temperature has effect on the growth of bacterial cells as well. In psychrotrophic microorganisms, essential cellular components like enzymes are thermolabile, hence these microorganisms conquer a lower optimum temperature of growth. *J. lividum* grows well in the temperature range of 20-25^{o}C. Maximum violacein production from *J. lividum* was observed at 25^{o}C followed by observation at 20^{o}C[49]. *Janthinobacterium* sp. strains were isolated growing in the temperature range of 2- 28^{o}C. Thus, violacein production was evident at 4^{o}C. Reports stated that the low temperature favours the violacein production[31]. No pigment production was observed at temperature of 30^{o}C and above[31,49]. However, *C. violaceum* strain ATCC 553 showed violacein production at temperature of 37^{o}C and 30^{o}C, the latter being higher [35]. Unlike pH, temperature does not affect the stability of the pigment. When heated for 2hrs at temperature range of 40-100^{o}C, violacein showed good stability[93].

Incubation time: Being a secondary metabolite, production of violacein is evident in the late log or stationary phase of cell growth. Violacein pigment production was observed when cultures were incubated for 16- 24 hrs with maximum production in between 16-18 hrs of incubation period[1,35]. Five days of incubation for *J. lividum* were found to be suitable for more violacein production[31]. Maximum production of violacein was reported after 24 hrs. of incubation by *Duganella violaceinigra* str. NI28 [29]. Similarly, *Massilia* sp. BS-1 shows violacein production after 24 hrs. incubation[1].

Rate of agitation: Rate of agitation could be one of the important factors affecting the production of pigment. Genes for violacein production are present in a *vio* operon. Violacein genes are regulated by quorum sensing associated with cell population[60]. *P. luteoviolacea* showed best violacein production at 0 rpm, showing higher bacterial biomass of cultures agitated at 100 rpm. It showed yellow colour in broth culture when incubated for 8 days at 200 rpm[94]. Violacein production by *J. lividum* enhanced when grown at agitation speed of 150 rpm [31]. *C. violaceum* strain ATCC 553 showed violacein production when grown at 200 rpm[35]. *Massilia* sp. BS-1showed violacein production when grown at the culture conditions maintaining agitation rate of 300 rpm[1].

Apart from these, some other factors also affect the violacein production. It is evident that tryptophan is essential for the violacein production. For conversion of tryptophan into violacein, molecular oxygen is required[35]. Important violacein producing bacteria, *J. lividum* and *C. violaceum* are aerobic.

Presence of oxygen is thus essential for the cell growth. Antibiotics are reported to have effect on violacein production. Antibiotics creates stressful environment leading to the production of violacein. Tetracycline inhibited

the rate of violacein production by 40% and tyrothrycin reduces the rate of violacein production by 90% at a concentration of 67µg/ml.

Penicillin levels from 0-200 µg/ml did not show any effect on growth of *C. violaceum* or on the violacein production after 24 hrs incubation[35]. Violacein production by *J. lividum* was found to be maximised when treated with 0.1-0.2mg/ml ampicillin. This antibiotic concentration did not influence the cell growth significantly. Further increase in the concentration of ampicillin (0.3-0.4 mg/ml), decreased cell growth but increased violacein production[49].

Applications

Antibacterial activity: Violacein proves a promising candidate in the current age of emergence of antibiotic resistant microorganisms. Crude violacein obtained from *D. violaceinigra* str. NI28 showed MIC of 15 µM for five multidrug resistant strains of *S. aureus* in Mueller-Hinton medium. Gram negative bacteria *P. aeruginosa* and *K. pneumoniae* were not inhibited by *D. violaceinigra* str. NI28 crude violacein[29]. *S. aureus* ATCC 3359 and *S. aureus* CCBH 5330 resistant to oxacillin were inhibited by violacein at the MIC of 20 µg/ml and 5 µg/ml respectively. Violacein also showed inhibitory effect on biofilm formation of *S. aureus* at the concentration of 1.25 µg/ml[3]. Ethanolic extracts of violacein from *Janthinobacterium* sp. SMN 33.6 showed antibacterial activity against carbapenemase producing *Acinetobacter baumannii* strain, *Pseudomonas aeruginosa* strain, extended-spectrum β-lactamases producing two strains of *E. coli*, two strains of *Klebsiella pneumoniae* and a chromosomal AmpC beta-lactamase producing two multi-resistant strains of *Serratia marcescens* with MIC values ranging between 0.5 and 16 µg/ml[8].

Ethanolic extracts of violacein from *Chromobacterium violaceum* (MTRI7) showed antibacterial activity against *B. subtilis, S. aureus, E. coli, S. typhi* and *K. pneumonia*[72]. Violacein from *C. violaceum* exhibited synergistic effect with penicillin G procaine against *S. aureus* isolates MBSA 24, 35 and 63, isolated from bovine mastitis[59]. Antimicrobial activity of violacein against attenuated *Mycobacterium tuberculosis* (H37Ra) with MIC of 64 µg/ml and MBC of 128 µg/ml was reported *in vitro*[39]. Violacein exhibited antimicrobial activity on 11 plant pathogens including *Erwinia carotovora, Pseudomonas cichorii, Colletotrichum dematium* and *Rosellinia necatrix*[80].

Violacein also showed either additive or synergistic effects when mixed in 1:1 ratio with different antibiotics against pathogenic organism *Salmonella typhi, Vibrio cholerae, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus.* Very few antibiotics were reported to exhibit antagonistic activity when used in combination with violacein [82]. The combination of violacein with Aztreonam, Cefuroxime, Meropenem and Ampicillin was found to be synergistic while with Ceftazidime it showed additive effect against uropathogenic *E. coli*[36]. Violacein from *Chromobacterium violaceum* displayed bactericidal and bacteriostatic action against *S. epidermidis* ATCC 12228 and *S. epidermidis* ATCC 35984 with MIC of 10 µg/ml and 20 µg/ml respectively. It also exhibited synergistic and additive effect with reduction in MIC from 4 -16-fold of antibiotics[37].

Antiviral Activity: Violacein showed antiviral activity against herpes simplex and Polio viruses. 62% HSV replication was inhibited by 0.25 µg/ml violacein and 0.063 µg/ml violacein inhibited 56% of polio virus replication in HeLa cells[41]. Contrary to these results, violacein exhibited weak inhibitory action on HSV-1 (strains KOS and ATCC/VR733) and PV-2 replication. Cytotoxic effect of violacein on different cell lines can be evident with trypan blue dye exclusion method and MTT assay. Violacein containing liposomes displayed increased antiviral activity against HSV-1[4].

Antifungal Activity: Pure violacein obtained from *Chromobacterium* sp. NIIST (MTCC 5522) was highly effective against human and plant pathogenic fungi. The eight fungi tested were *A. flavus, C. albicans, C. tropicalis, C. gastricus, T. rubrum, R. solani, F. oxysporum* and *P. expansum.* Out of these, violacein was found more effective against *A. flavus, R. solani, F. oxysporum* and *P. expansum* as compared to the common antifungal agents bavistin and amphotericin B [78]. Crude violacein extracts recovered from *J.lividum* exhibited antifungal activity against phytopathogenic fungi *Colletitrichum dematium* and *Rosellinia necatrix* causing anthracnose and white root-rot diseases of mulberry respectively[39]. Brazilian strains of *C.violaceum* were found effective against seed pathogenic fungi *Fusarium* sp., *Phomopsis* sp. and *Cercospora kikuchi*. The strains also exhibited antifungal activity against *Corynespora* sp., *Aspergillus* sp. and *Colletotrichum* sp. The antifungal activity is attributed to the pigment violacein, chitinases and cyanide production by these strains[15].

Violacein from *J. lividum* displayed antifungal activity against *Batrachochytrium dendrobatidis* (Bd) which causes chytridiomycosis, a major factor responsible for extinction of amphibian species [44,86]. Out of the six *R. muscosa* juveniles frogs tested, only one bioaugmented with *J. lividum* survived in the study. Five juveniles not treated with *J. lividum* developed skin infection chytridiomycosis and died. These results suggest higher population of *J. lividum* leading to the violacein production which exhibited antifungal activity[44].

Anticancer Activity: Violacein was reported as an anticancer molecule. When tested on human colorectal cancer, HCT116 cells by CI-isobologram analysis, violacein exhibits growth inhibitory effect in synergism with 5-fluorouracil. Quantification of MTT assay by flow cytometry indicates a dose dependent increase in the apoptosis of HCT116 cells and enhanced apoptosis by 5-FU. Violacein presented combined cytostatic and cytotoxic

effects along with 5-FU on HCT116 cells. Western blot analysis of proteins extracted from HCT116 cells treated with 1µM violacein displayed time-dependent upregulation of p21, p27 and p53 protein expression and a significant reduction of cyclin D1 expression along with pRb downregulation. Further violacein induces apoptosis in cancer cells, evident by increased levels of pro apoptotic Bad proteins and decreased levels in the phosphorylated Bad^{ser136}. Violacein acts by preventing Akt (Protein kinase B) with subsequent inhibition of NFκB translocation by blocking IκBα phosphorylation[50].

Violacein extracted from recombinant *E. coli* and *Salmonella typhimurium* strain VPN20009 showed inhibitory action on proliferation of A549 non-small cell lung cancer, A431 Melanoma, MCF-7 breast cancer, PC3 prostate cancer, HT29 colon cancer and HeLa cervical cancer cells with IC_{50} values ranging from 45-352 nM. It was also found effective against HCT116 colon and HN5 head and neck cancer. MTT proliferation assay of cells cultured in a GasPak EZ pouch displayed that hypoxia sensitized all cancer cells to violacein. Ethyl acetate and ethanol crude extracts obtained from five Brazilian isolates of *Chromobacterium* sp showed *in vitro* antitumor activity against central nervous system cancer, breast cancer, melanoma, ovarian cancer expressing a multiple drug resistance phenotype, renal cancer, small cell lung carcinoma, prostate cancer, ovarian cancer, colon cancer and *in vivo* in Ehrlich solid carcinoma in mice.

HPLC-diode array detection analysis detected presence of violacein and deoxyviolacein in all the extracts, highlighting the potential of this secondary metabolite to act as an antiproliferative agent in cancer treatment[61]. Violacein obtained from transformed *E. coli* BL21(DE3) cells with plasmids carrying genes *vioABCDE* displayed cytotoxicity on HepG2 (human hepatoma) and COS-7(kidney from African Green Monkey) cell lines with IC_{50} of ~1.4 µM and ~ 2.5 µM respectively[16].

Violacein induced caspase3/7 activity in A549 non-small cell lung cancer cells. Further head and neck squamous carcinoma subcutaneous xenograft models established in BALB/c nude mice exhibited regression of tumors when treated with violacein dose of 0.7 mg/kg of body weight. Violacein also slowed the growth rate of tumors. Violacein treated animals did not differ from control in terms of body weight, behavior or phenotypic characters. This data has been apparent with *in vivo* antitumor activity of violacein[45]. Violacein loaded dendrimer with ascorbic acid exhibited increase in the caspase 3, 7 and 8 activities by 3.5, 4.5 and 6 times respectively in the human acute lymphoblastic leukemia Jurkat E6.1 cell line[42].

Violacein showed decrease in the human leukemia cell proliferation with IC50 value of less than 1 µmol/L. One of the important components of cell signaling control is protein phosphatases. The inhibitory effects were attributed to the modification of sulfhydryl groups of protein tyrosine phosphatase. Another possibility thought for violacein cytotoxic effect is of hydrophobic interactions between violacein and enzyme[18]. Violacein from *C. vaccinii* CV5 displayed cytotoxicity on HeLa (cervical cancer cell) and A549 (lung cancer cell) cell lines with IC50 values of 26 µg/mL and 31 µg/mL respectively[77].

Direct and specific activation of violacein on TNF receptor I signaling in HL60 cells makes it a member of novel class of cytotoxic drug mediating apoptosis. Violacein influenced morphological changes typical of apoptosis in human myeloid leukemia cells[41].

Matrix metalloproteinases (MMP) play important role in cancer metastasis. MCF-7 breast cancer cells treated with 1µM violacein in DMSO down modulated expression of MMP-2 from 156 ± 22 to 42 ±10 (densitometric units) $p<0.05$. This is credited to the anti-inflammatory effect of violacein. There is a correlation between cancer metastasis and chemokine CXCL12 and its interaction with receptor CXCR4. Direct inhibition of pro-inflammatory cytokine induced MMP-2 mediated CXCL12 secretion was displayed in violacein treated MCF-7 breast cancer cells. Violacein also demonstrated to inhibit MMP-9 expression and activity efficiently[69].

Violacein reported to cause programmed cell death in HT29 and Caco-2 human colon cancer cells by producing reactive oxygen species (ROS). The excessive ROS leads to cell death by elevating calcium level which further impairs mitochondrial function which in turn may activate enzymes like endonucleases, proteases, phospholipases leading to the irreversible damage to organelles, membrane and chromatin causing cell death[33].

$CD34^+$/c-Kit^+/P-$glycoprotein^+$/$MRP1^+$ TF1 leukemia progenitor cells are usually reported to be resistant against PCD found to be sensitive to violacein in MTT reduction assay. Violacein induces oxidative stress in these cells which leads to the linearization of endoplasmic reticulum and Golgi bodies. Kinases like CDK, Rock, Axl and AurkA which promote cell cycle progression and cell survival were negatively modulated by violacein as revealed by Kinome profiling. Violacein modulated two kinases linked with reticulum stress and cell death by apoptosis. It increases autophosphorylation of DAPK1 at its inhibitory site and leads to expressive activation of PKA by causing higher phosphorylation of its substrate CREB1[70].

Antiparasitic Activity: *In vitro* and *in vivo* antiplasmodial activities were exhibited by violacein. It showed IC_{50} of 0.85 ± 0.11 µM against *P. falciparum* strain 3D7. The lowest tested concentration of violacein of 0.06 µM was effective in inhibition of parasite development after 48 hr. of incubation. C57BL/6 mice infected with a nonlethal (AS) *Plasmodium chabaudi chabaudi* used for *in vivo* assay showed 39% inhibition in parasitaemia on day 7 post

infection even at low dose of 0.75 mg/kg/day. Moreover, animals infected with the lethal AJ strain of *P. chabaudi chabaudi* treated with 7.5 mg/kg/day violacein showed 80% survival on day 16 post infection, significantly demonstrating protective effect of violacein[55].

Violacein obtained from transformed *E. coli* BL21(DE3) cells with plasmids carrying genes *vioABCDE* was found to efficiently inhibit trypanosomatids (*T. cruzi*) with IC_{50} of 1.51 μM ± 0.4. This value was lower than the 3.07 μM ± 0.6, IC_{50} of anti *T. cruzi* drug benzimidazole, highlighting the typanocidal activity of violacein. It also displayed anti plasmodial activity against 3D7 (wild-type) and W2 (chloroquine-resistant) *Plasmodium falciparum* strains with IC_{50} ~ 0.4 μM against 3D7 and ~ 0.5 μM against W2 parasites[16]. Violacein glucoside derivatives showed anti-nematode activity against pine wood nematode *Bursaphelenchus xylophilus*[54].

Other biological Activities: TNF-α mRNA expression was induced by violacein at a concentration of 2μmol/L or higher in Raw 264.7 and ANA-1 cells with stimulation index of 1.5 for Raw 264.7 cells and 3.6 in ANA-1 cells. Incubation of Raw 264.7 cells with violacein shown significant change in genes expression. DAVID databases analysis displayed violacein induced changes in the expression of genes involved in the immune and inflammatory response pathways, regulation of cell proliferation and apoptotic pathway. Cytotoxic effect was showed by violacein on HEK 293 cells transfected with hTLR7 with no NFκB induction at concentration of 15 and 30 μmol/L. These result points towards the immunostimulatory activity of violacein [91].

Violacein demonstrated the possibility to control autoimmune encephalomyelitis (EAE) with the initiation of regulatory T cells (Treg), regulation of inflammatory modulators like IL-10, IL-17, indoleamine 2,3-dioxygenase and dendritic cells[48,92].

Proinflammatory cytokines like TNF-α, IL-1β, IL-6, anti-inflammatory cytokines such as IL-4, IL-10 and growth factors vascular endothelial growth factor (VEGF), endothelial growth factor (EGF) and hepatocyte growth factor (HGF) have important roles in modulating ulcer healing. These factors increase angiogenesis and gastric mucin and decrease gastric acid secretion.

Violacein exhibited gastroprotective effect in indomethacin-induced gastric ulcer rat model by reducing proinflammatory cytokines and increasing anti-inflammatory cytokines and growth factors[6,48].

Partially purified violacein from *Chromobacterium vaccinii* CV5 displayed antioxidant activity against free radicals DPPH (1, 1-Diphenly2picryl-hydrozil), nitric oxide, superoxide, hydroxyl and hydrogen peroxide with IC_{50} values of 297.88 μg/mL, 312.89 μg/mL, 410.17μg/mL and 296.74 μg/mL respectively. This result highlights the potential of violacein as a potent free radical scavenger and hence applicability in pharmaceutical industry[84].

Denaturing gradient gel electrophoresis (DGGE) and non-metric dimensional scaling (NMDS) analyses revealed change in the composition of gut microbiota with the administration of 50 μg/mL and 500 μg/mL of violacein daily for a month in two-month old male Wistar albino rats[67].

Industrial Applications: Apart from the different biological activities, violacein is a vital candidate for food and textile industries. Methanolic extracts of violacein and deoxyviolacein obtained from *J. lividum* gave purple to dark blue colour shades to natural and synthetic fibres like silk, cotton, wool, nylon, acetate and vinylon with comparable colour fastness to vegetable dyes. Further, treatment with thiourea post dyeing reduced the fading of colour due to sunlight[80]. Similar result was obtained with violacein extracted from *C. violaceum* UTM5 with pure cotton, pure silk, pure rayon, jacquard rayon, silk satin, cotton and polyester. $Fe_2(SO_4)_3$ and $CuSO_4$ increased lightfastness of cotton fibre whereas alum and slaked lime increased lightfastness of silk satin. Slaked lime also improved the colour shade when used as a mordant, but the colour shades depend upon the type of fibre[25].

The antibacterial and antioxidant properties of violacein were exploited by incorporation of this bisindole dye into cosmetics. Violacein was proposed to be used in the preparation of consumer and environment friendly products of toy, food and textile industry [39,79]. There was an hyperchromic effect of different pH on crude violacein extracted from *C. violaceum* MTCC 2656. This could be evident from shift towards longer wavelength in acidic and alkaline condition along with change in the color of the pigment. Further studies are required to explore this fact to use violacein as a pH indicator[64].

Conclusion

Violacein is produced by vio operon comprising of *vio ABCDE* genes. Out of these genes *VioB, VioC* and *VioD* are more important than *VioA* and *VioE* in the violacein production. The control of a diffusible signal molecule named N-acyl-homoserine-lactone over biosynthesis of violacein validates more pigment production in the stationary phase as N-acyl-homoserine-lactone is involved in quorum sensing. Increased violacein production is reported in media supplemented with slow consumption carbon sources like sucrose and tryptophan or having higher amino acid content. pH 7 was found to be good for the pigment production. Optimum temperature depends on the bacteria producing it. Media optimization by using cheap raw material to produce violacein will reduce the cost of production suitable for industrial applications.

Violacein appeared to be a potent pharmaceutical product. This bisindole pigment has shown potential therapeutic applications *in vitro* and *in vivo*. It holds promise as a

therapeutic agent for the treatment of infectious diseases. This accounts to the antibacterial, antifungal, antiviral and antiparasitic activity shown by this secondary metabolite. Violacein displayed immunomodulatory activity which could be used for the treatment of autoimmune diseases. Above all these properties, its greatest potential lies in the anticancer properties. Violacein exhibited anticancer activity against different types of cancers. This makes it a potential pharmaceutical candidate. Apart from pharmaceutical industry, violacein has various applications in textile, cosmetics and food industry as a coloring agent with properties like antioxidant. Use of nanotechnology will be beneficial to the applications of this pigment in near future.

Earlier reviews published on the violacein highlighted various applications of this bacterial secondary metabolite. This review is an effort to compile the information about some important aspects of violacein, a promising bacterial secondary metabolite.

References

1. Agematu H., Suzuki K. and Tsuya H., *Massilia* sp. BS-1, a Novel Violacein-Producing Bacterium Isolated from Soil, *Bioscience, Biotech and Biochem*., **75(10)**, 2008–2010 **(2011)**

2. Ahmad W.A., Wan Ahmad W.Y., Zakaria Z.A. and Yusof N.Z. Applications of Bacterial Pigments as Colorant, The Malaysian Perspective, Springer, Berlin, Heidelberg, 57-74 **(2015)**

3. Andressa H.M. et al, Antimicrobial Effects of Violacein against Planktonic Cells and Biofilms of *Staphylococcus aureus*, *Molecules*, **22**, 1534 **(2017)**

4. Andrighetti-Frohner C.R., Antonio R.V., Creczynski-Pasa T.B., Barardi C.R.M. and Simoes C.M.O., Cytotoxicity and Potential Antiviral Evaluation of Violacein Produced by *Chromobacterium violaceum*, *Mem Inst Oswaldo Cruz*, **98(6)**, 843-848 **(2003)**

5. Antonio R.V. and Creczynski-Pasa T.B., Genetic analysis of Violacein Biosynthesis by *Chromobacterium violaceum, Genetics and Mol. Research*, **3(1)**, 85-91 **(2004)**

6. Antonisamy P., Kannan P., Aravinthan A., Duraipandiyan V., Arasu M.V., Ignacimuthu S., Al-Dhabi N.A. and Kim J.H., Gastroprotective Activity of Violacein Isolated from *Chromobacterium violaceum* on Indomethacin-Induced Gastric Lesions in Rats: Investigation of Potential Mechanisms of Action, *Sci. World J*., doi: 10.1155/2014/616432 **(2014)**

7. Aruldass C.A., Rubiyatno, Chidambaram K.V. and Wan A.A., Violet Pigment Production from Liquid Pineapple Waste by *Chromobacterium violaceum* UTM5 and Evaluation of its Bioactivity, *Royal Soc. of Chem. Adv*., **5**, 51524–51536 **(2015)**

8. Asencio G. et al, Antibacterial Activity of the Antarctic Bacterium *Janthinobacterium* sp. SMN 33.6 Against Multi-Resistant Gram-Negative Bacteria, *Electronic J. of Biotech*., **17**, 1–5 **(2014)**

9. Atalah J., Blamey L., Munoz-Ibacache S., Gutierrez F., Urzua M., Encinas M.V., Paez M., Sun J. and Blamey J.M., Isolation and Characterization of Violacein from an Antarctic *Iodobacter*: A Non-Pathogenic Psychrotolerant Microorganism, *Extremophiles*, **24**, 43–52 **(2020)**

10. August P.R., Grossman T.H., Minor C., Draper M.P., MacNeil I.A., Pemberton J.M., Call K.M., Holt D. and Osburne M.S., Sequence Analysis and Functional Characterization of the Violacein Biosynthetic Pathway from *Chromobacterium violaceum*, *J. Mol. Micro. Biotech*, **2(4)**, 513-519 **(2000)**

11. Aye A.M., Jusserand M.B., Jaisson F.B., Magne A.O., Culioli G., Nevry R.K., Rabah N., Blache Y. and Molmeret M., Modulation of Violacein Production and Phenotypes Associated with Biofilm by Exogenous Quorum Sensing N-acylhomoserine Lactones in the Marine Bacterium *Pseudoalteromonas ulvae* TC14, *Microbiology*, **161**, 2039–2052 **(2015)**

12. Azamjon B.S., Hosokawa K. and Enomoto K., Bioactive Pigments from Marine Bacteria: Applications and Physiological Roles, *Evidence-Based Complementary and Alternative Med*., **2011**, 670349 **(2011)**

13. Azman A.S., Mawang C.I. and Abubakar S., Bacterial Pigments: The Bioactivities and as an Alternative for Therapeutic Applications, *Natural Product Communications*, **13(12)**, 1747-1754 **(2018)**

14. Baricz A., Teban A., Chiriac C.M., Szekeres E., Farkas A., Nica M., Dascalu A., Oprișan C., Lavin P. and Coman C., Investigating the Potential Use of an Antarctic Variant of *Janthinobacterium lividum* for Tackling Antimicrobial Resistance in A One Health Approach, *Scientific Reports*, **8**, 15272 **(2018)**

15. Barreto E.S., Torres A.R., Barreto M.R., Vasconcelos A.T.R., Filho S.A. and Hungria M., Diversity in antifungal activity of strains of *Chromobacterium violaceum* from the Brazilian Amazon, *J Ind Microbio. Biotech*, **35**, 783–790 **(2008)**

16. Bilsland E., Tavella T.A., Krogh R., Stokes J.E., Roberts A., Spring J.D., Andricopulo A.D., Costa F.T.M. and Oliver S.G., Antiplasmodial and Trypanocidal Activity of Violacein and Deoxyviolacein Produced from Synthetic Operons, *BMC Biotech*, **18(22)**, DOI: 10.1186/s12896-018-0428-z **(2018)**

17. Bromberg N. and Duran N., Violacein Biotransformation by Basidiomycetes and Bacteria, *Letters in Appl. Micro*., **33**, 316-319 **(2001)**

18. Bromberg N., Justo G.Z., Haun M., Duran N. and Ferreira C.V., Violacein cytotoxicity on human blood lymphocytes and effect on phosphatases, *J. of Enzyme Inhibition and Med. Chem*., **20(5)**, 449-454 **(2005)**

19. Burt S.A., Ojo-Fakunle V.T.A., Woertman J. and Veldhuizen E.J.A., The Natural Antimicrobial Carvacrol Inhibits Quorum Sensing in *Chromobacterium violaceum* and Reduces Bacterial Biofilm Formation at Sub-Lethal Concentrations, *PLoS ONE*, **9(4)**, e93414 **(2014)**

20. Cardona-Cardona V., Arroyo D., Scellekens J. and Rios-Velazquez C., Characterisation of Blue Pigmented Bacteria Isolated from Puerto Rico, Current Research, Tech. and Edu. Topics in Appl. Micro. and Microbial Biotech., Mendez-Vilas ed., 117-123 **(2010)**

21. Charkoudian L.K., Fitzgerald J.T., Khosla C. and Champlin A., In Living Color: Bacterial Pigments as an Untapped Resource in the Classroom and Beyond, *PLoS Bio*., **8(10)**, e1000510 **(2010)**

22. Chatragadda R., Nambali V.V. and Ramalingam K., Marine Pigmented Bacteria: A Prospective Source of Antibacterial Compounds, *J. of Natural Sci., Bio. and Med*., **10(2)**, 104-113 **(2019)**

23. Chatragadda R., Nambali V.V., Ramalingam K., Chidambaram K.V. and Laurent D., Multifaceted Applications of Microbial Pigments: Current Knowledge, Challenges and Future Directions for Public Health Implications, *Microorganisms*, **7**, 186 **(2019)**

24. Chaudhari S., Mehta M. and Shah G., Extraction of Plant and Microbial Pigment, Comparison and to Check Its Stability, *J. of Emerging Tech. and Innovative Research*, **6(6)**, 184-190 **(2019)**

25. Chidambaram K.V., Nur Z.Y., Claira A.A. and Wan A.A., Application of violet pigment from *Chromobacterium violaceum* UTM5 in textile dyeing, *Cellular and Mol. Bio.*, DOI: 10.1515/biolog-2016-0031 **(2016)**

26. Chidambaram K.V. and Perumalsamy L., An Insightful Overview on Microbial Pigment, Prodigiosin, *Electron. J. Biotechnol*., **5(3)**, 49-61 **(2009)**

27. Chidambaram K.V., Zakaria Z.A., Rajamanickam U. and Wan A.A., Isolation and Characterization of Flexirubin Type Pigment from *Chryseobacterium* sp. UTM-3T, *Biocatalysis and Agri. Biotech*., **3**, 103–107 **(2014)**

28. Chidambaram K.V., Zakaria Z.A. and Wan A.A., Bacterial pigments and their applications, *Process Biochem*., **48**, 1065–1079 **(2013)**

29. Choi S.Y., Kim S., Lyuck S., Kim S.B. and Mitchell R.J., High-level production of violacein by the newly isolated *Duganella violaceinigra* str. NI28 and its impact on *Staphylococcus aureus*, *Scientific Reports*, **5**, 15598 **(2015)**

30. Choi S.Y., Yoon K., Lee J.I. and Mitchell R.J., Violacein: Properties and Production of a Versatile Bacterial Pigment, *Bio Med Research Int*., **2015**, 465056 **(2015)**

31. Cortes-Osorio N., Cardoso-Cardoso M., Chavarro-Anzola V., Prada-Salcedo L.D., Reyes-Cardenas A., Robles-Camargo J. and Franco-Correa M., Influence of Environmental Factors on the Production of Violacein Synthesized by *Janthinobacterium lividum*, *The Int. J. of Eng. and Sci.*, **6(1)**, 76-83 **(2017)**

32. Dang H.T., Yotsumoto K. and Enomoto K., Draft Genome Sequence of Violacein-Producing Marine Bacterium *Pseudoalteromonas* sp. 520P1, *Genome Announce*, **2(6)**, DOI:10.1128/genomeA.01346-14e **(2014)**

33. Daniela D., Carvalho D., Costa F.T.M., Duran N. and Haun M., Cytotoxic Activity of Violacein in Human Colon Cancer Cells, *Toxicology in Vitro*, **20**, 1514–1521 **(2006)**

34. Deines C.M., Boenigk J., Arndt H., Eberl L., Kjelleberg S. and Jurgens K., Impact of Violacein-Producing Bacteria on Survival and Feeding of Bacterivorous Nanoflagellates, *Appl. and Env. Micro*., **70(3)**, 1593–1599 **(2004)**

35. Demoss R.D. and Evans N.R., Physiological Aspects of Violacein Biosynthesis in Nonproliferating Cells, *J. Bacteriol*, **78**, 583-588 **(1959)**

36. Devi P., Kannan S.S.R. and Mariappan T.K., Production of Violacein Pigment from *Chromobacterium violaceum* and its Antibacterial Activity and Synergism on *E. coli* Isolated from UTI Samples, *Int. J. of Recent Scientific Research*, **9(2)**, 24479-24484 **(2018)**

37. Dodou H.V., de Morais Batista A.H., Sales G.W.P., de Medeiros S.C., Rodrigues M.L., Nogueira P.C.N., Silveira E.R. and Nogueira N.A.P., Violacein Antimicrobial Activity on *Staphylococcus epidermidis* and Synergistic Effect on Commercially Available Antibiotics, *J. of Appl. Micro*., **123**, 853—860 **(2017)**

38. Doing G., Perron G.G. and Brooke A.J., Draft Genome Sequence of a Violacein-Producing *Iodobacter* sp. From the Hudson Valley Watershed, *American Soci. for Micro*., **6(1)**, e01428 **(2018)**

39. Duran M., Ponezi A.N., Faljoni-Alario A., Teixeira M.F.S., Justo G.Z. and Duran N., Potential Applications of Violacein: A Microbial Pigment, *Med Chem Res*, **21**, 1524–1532 **(2012)**

40. Duran N., Justo G.Z., Duran M., Brocchi M., Cordi L., Tasic L., Castro G.R. and Nakazato G., Advances in *Chromobacterium violaceum* and Properties of Violacein-Its Main Secondary Metabolite: A Review, *Biotech Advances*, **34**, 1030–1045 **(2016)**

41. Duran N., Justo G.Z., Ferreira C.V., Melo P.S., Cordi L. and Martins D., Violacein: Properties and Biological Activities, *Biotech. Appl. Biochem*., **48**, 127–133 **(2007)**

42. Fakhr F.A., Khanafari A., Baserisalehi M., Yaghoobi R. and Shahghasempour S., An Investigation of Antileukemia Activity of Violacein-Loaded Dendrimer in Jurkat Cell Lines, *African J. of Micro. Research*, **6(33)**, 6235-6242 **(2012)**

43. Hakvag S., Fjærvik E., Klinkenberg G., Borgos S.E.F., Josefsen K.D., Ellingsen T.E. and Zotchev S.B., Violacein-Producing *Collimonas* sp. from the Sea Surface Microlayer of Coastal Waters in Trondelag, Norway, *Marine Drugs*, **7**, 576-588 **(2009)**

44. Harris R.N., Brucker R.M., Walke J.B., Becker M.H., Schwantes C.R., Flaherty D.V., Lam B.A., Woodhams D.C., Briggs C.J., Vredenburg V.T. and Minbiole K.P.C., Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus, *The Int. Soci. for Micro. Ecology J*., **3**, 818–824 **(2009)**

45. Hashimi S.M., Xu T. and Wei M.Q., Violacein anticancer activity is enhanced under hypoxia, *Oncology Reports*, **33**, 1731-1736 **(2015)**

46. Hassaan M.A. and Nemr A.E., Health and Environmental Impacts of Dyes: Mini Review, *American J. of Env. Sci. and Eng.*, **1(3)**, 64-67 **(2017)**

47. Hizbullahi M.U., Nafiu A., Gani M. and Maiturare H.M., Bacterial Pigments and its Significance, *MOJ Bioequi. & Bioavail*., **4(3)**, 285-288 **(2017)**

48. Joshi V.K., Attri D., Bala A. and Bhushan S., Microbial Pigments, *Indian J. of Biotech.*, **2**, 362-369 **(2003)**

49. Justo G.Z. and Duran N., Action and Function of *Chromobacterium violaceum* in Health and Disease: Violacein as a Promising Metabolite to Counteract Gastroenterological Diseases, *Best Practice & Research Clin. Gastroenterology*, **31**, 649-656 **(2017)**

50. Kanelli M., Mandic M., Kalakona M., Vasilakos S., Kekos D., Nikodinovic-Runic J. and Topakas E., Microbial Production of Violacein and Process Optimization for Dyeing Polyamide Fabrics with Acquired Antimicrobial Properties, *Frontiers in Micro*., **9**, 1495 **(2018)**

51. Kodach L.L., Bos C.L., Duran N., Peppelenbosch M.P., Ferreira C.V. and Hardwick J.C.H., Violacein synergistically increases 5-fluorouracil cytotoxicity, induces apoptosis and inhibits Akt-mediated signal transduction in human colorectal cancer cells, *Carcinogenesis*, **27(3)**, 508–516 **(2006)**

52. Konzen M., Marco D.D., Cordova C.A.S., Vieira T.O., Antonio R.V. and Creczynski-Pasa T.B., Antioxidant Properties of Violacein: Possible Relation on Its Biological Function, *Bioorganic & Med. Chem*., **14**, 8307–8313 **(2006)**

53. Kothari V., Sharma S. and Padia D., Recent research advances on *Chromobacterium violaceum*, *Asian Pacific J. of Trop. Med*., **10(8)**, 744–752 **(2017)**

54. Kumar A., Vishwakarma H.S., Singh J., Dwivedi S. and Kumar M., Microbial Pigments: Production and Their Applications in Various Industries, *Int. J. of Pharma., Chemical and Bio. Sci*., **5(1)**, 203-212 **(2015)**

55. Lee Y.J., Bashyal P., Pandey R.P. and Sohng J.K., Enzymatic and Microbial Biosynthesis of Novel Violacein Glycosides with Enhanced Water Solubility and Improved Anti-nematode Activity, *Biotech. and Bioprocess Eng.*, **24**, 366-374 **(2019)**

56. Lopes S.C.P., Blanco Y.C., Justo G.Z., Nogueira P.A., Rodrigues F.L.S., Goelnitz U., Wunderlich G., Facchini G., Brocchi M., Duran N. and Costa F.T.M., Violacein Extracted from *Chromobacterium violaceum* Inhibits *Plasmodium* Growth *In Vitro* and *In Vivo*, *Antimicrobial Agents and Chemo.*, **53(3)**, 2149-2152 **(2009)**

57. Gulrajani M.L., Present Status of Natural Dyes, *Indian J. of Fibre and Textile Research*, **26**, 191-201 **(2001)**

58. Prasad M.P., Optimization of media parameters for pigment production in bacteria from effluent water samples, *Biolife*, **3(2)**, 428-433 **(2015)**

59. Malik K., Tokkas J. and Goyal S., Microbial Pigments: A Review, *Int. J. of Microbial Resource Tech*., **1(4)**, 361-365 **(2012)**

60. Martins D., Ribeiro M.G., Duran N. and Nakazato Gerson, Antibacterial Activity of Violacein against *Staphylococcus aureus* Isolated from Bovine Mastitis, *The J. of Antibiotics*, **64**, 395-397 **(2011)**

61. McClean K.H., Winson M.K., Fish L., Taylor A., Chhabra S.R., Camara M., Daykin M., Lamb J.H., Swift S., Bycroft B.W., Stewart G.S.A.B. and Williams P., Quorum sensing and *Chromobacterium violaceum*: Exploitation of Violacein Production and Inhibition for the Detection of N-acyl Homoserine Lactones, *Microbiology*, **143**, 3703-3711 **(1997)**

62. Menezes C.B.A., Silva B.P., Sousa I.M.O., Ruiz A.L.T.G., Spindola H.M., Cabral E., Eberlin M.N., Tinti S.V., Carvalho J.E., Foglio M.A. and Fantinatti- Garboggini F., *In Vitro* and *In Vivo* Antitumor Activity of Crude Extracts Obtained from Brazilian *Chromobacterium* sp Isolates, *Brazilian J. of Med. and Bio. Research*, **46**, 65-70 **(2013)**

63. Mohammadi M., Burbank L. and Roper C.M., Biological Role of Pigment Production for the Bacterial Phytopathogen *Pantoea stewartii* subsp. *Stewartia, Appl. and Envi. Micro*., **78(19)**, 6859-6865 **(2012)**

64. Morohoshi T., Fukamachi K., Kato M., Kato N. and Ikeda T., Regulation of the Violacein Biosynthetic Gene Cluster by Acylhomoserine Lactone-Mediated Quorum Sensing in *Chromobacterium violaceum* ATCC 12472, *Biosci., Biotech. and Biochem*., **74(10)**, 2116–2119 **(2010)**

65. Nathan V.K., Subha Rajam K., Rani M.E., Rathinasamy G. and Kannan N.D., Surface Culturing of *Chromobacterium violaceum* MTCC 2656 for Violacein Production and Prospecting its Bio-Activities, Chapter 3, Current Research in Microbiology **(2019)**

66. Numan M., Bashir S., Mumtaz R., Tayyab S., Rehman N.U., Khan A.L., Shinwari Z.K. and Harrasi A.A., Therapeutic Applications of Bacterial Pigments: A Review of Current Status and Future Opportunities, *3 Biotech*, **8**, 207 **(2018)**

67. Pantanella F., Berlutti F., Passariello C., Sarli S., Morea C. and Schippa S., Violacein and biofilm production in *Janthinobacterium lividum*, *J. of Appl. Micro*., **102**, 992–999 **(2007)**

68. Pauer H., Hardoim C.C., Teixeira F.L., Karla R.M., Barbirato D.S., de Carvalho D.P., Martha Antunes L.C., da Costa Leitao A., Lobo L.A. and Pilotto Domingues R.M.C., Impact of Violacein from *Chromobacterium violaceum* on the Mammalian Gut Microbiome, *PLoS ONE*, DOI: 10.1371/journal.pone.0203748 **(2018)**

69. Pereira L. and Alves M., Chapter 4 Dyes-Environmental Impact and Remediation, Environmental Protection Strategies for Sustainable Development, Strategies for Sustainability, Springer Sci., Business Media B.V., 11-154 **(2012)**

70. Platt D., Amara S., Mehta T., Vercuyssee K., Myles E.L., Johnson T. and Tiriveedhi V., Violacein inhibits matrix metalloproteinase mediated CXCR4 expression: Potential anti-tumor effect in cancer invasion and metastasis, *Biochem Biophys Res Commun*, **5(455)**, 107–112 **(2014)**

71. Queiroz K.C.S., Milani R., Ruela-de-Sousa R.R., Fuhler G.M., Justo G.Z., Zambuzzi W.F., Duran N., Diks H.S., Spek C.A., Ferreira C.V. and Peppelenbosch M.P., Violacein Induces Death of Resistant Leukaemia Cells via Kinome Reprogramming, Endoplasmic Reticulum Stress and Golgi Apparatus Collapse, *PLoS ONE*, **7(10)**, e45362 **(2012)**

72. Siva R., Status of Natural Dyes and Dye Yielding Plants in India, *Current Science*, **92(7)**, 916-925 **(2007)**

73. Rokade M.T. and Pethe A.S., Isolation, Identification, Extraction and Production of Antibacterial Violacein Pigment by Psychrotrophic Bacterium MTRI7 Strain, *J. of Global Biosci*., **6(6)**, 5077-5083 **(2017)**

74. Ruhul Momen Z.M. and Hoshino T., Biosynthesis of Violacein: Intact Incorporation of the Tryptophan Molecule on the Oxindole Side, with Intramolecular Rearrangement of the Indole Ring on the 5-Hydroxyindole Side, *Biosci., Biotech. and Biochem*., **64(3)**, 539-549 **(2000)**

75. Rymbai H., Sharma R.R. and Srivastav M., Biocolorants and its Implications in Health and Food Industry - A Review, *Int. J. of Pharm Tech Research*, **3(4)**, 2228-2244 **(2011)**

76. Samyukta S. and Naphade Mahajan S., Isolation and Identification of Pigment Producing Bacteria and Characterization of Extracted Pigment, *Int. J. of Appl. Research*, **2(7)**, 657-664 **(2016)**

77. Santhosh V.T. and Palaniswamy M., Evaluation of *in Vitro* Cytotoxic Effect of Violacein Produced by Novel Isolate *Chromobacterium vaccinii* CV5 Against the Cervical and Lung Cancer Cell, *Asian J. of Pharma. and Clinical Research*, **10(10)**, 227-229 **(2017)**

78. Santhosh V.T. and Palaniswamy M., Isolation and Identification of *Chromobacterium* Sp. from Different Ecosystems, *Asian J. of Pharma. and Clinical Research*, **9(Suppl, 3)**, 253-257 **(2016)**

79. Sasidharan A., Sasidharan N.K., Bhaskaran D., Nair S., Radhakrishnan K.V., Nataraja A.V. and Bhaskaran K., Antifungal activity of violacein purified from a novel strain of *Chromobacterium* sp. NIIST (MTCC 5522), *J. of Micro.*, **53(10)**, 694–701 **(2015)**

80. Sen T., Barrow C.J. and Deshmukh S.K., Microbial Pigments in the Food Industry - Challenges and the Way Forward, *Front. in Nutri.*, **6**, 7 **(2019)**

81. Shirata A., Tsukamo T., Yasuj H., Hata T., Hayasaka S.S., Kojima A.A. and Kato H., Isolation of Bacteria Producing Bluish-Purple Pigment and Use for Dyeing, *Japan Agri. Research Quarterly*, **34**, 131- 140 **(2000)**

82. Sinha S., Choubey S., Ajay Kumar A. and Bhosale P., Identification, Characterization of Pigment Producing Bacteria from Soil and Water and Testing of Antimicrobial Activity of Bacterial Pigments, *Int. J. of Pharma. Sci. Rev. Res*., **42(2)**, 119-124 **(2017)**

83. Subramaniam S., Venkatraman R. and Sivasubramanian A., Synergistic Antimicrobial Profiling of Violacein with Commercial Antibiotics Against Pathogenic Microorganisms, *Pharma. Biology*, **52(1)**, 86-90 **(2014)**

84. Suman R., Sharma P., Gupta S., Sourirajan A. and Dev K., A Novel Psychrophilic *Janthinobacterium lividum* MMPP4 Isolated from Manimahesh Lake of Chamba District of Himachal Pradesh, India, *J Biochem Tech*, **6(1)**, 846-851 **(2015)**

85. Vishnu T.S. and Palaniswamy M., Systematic Approach on Evaluating the *in vitro* Antioxidant Activity of Violacein; Novel Isolate *Chromobacterium Vaccinii* CV5, *Biomed. & Pharmaco. J*., **11(2)**, 703-709 **(2018)**

86. Tabor-Godwin J., Stuart R., Leon Zayas R.I. and Rajakuberan C., What you Didn't Know about *Janthinobacterium*, Physiology & Genetics, Teachers Corner **(2009)**

87. Thorpe C.J., Lewis T.R., Fisher M.C., Wierzbicki C.J., Kulkarni S., Pryce D., Lewis D., Watve A. and Knight M.E., Climate structuring of *Batrachochytrium dendrobatidis* infection in the threatened amphibians of the northern Western Ghats, India, *Royal Soc. Open Sci.*, **5**, 180211 **(2018)**

88. Tuli H.S., Chaudhary P., Beniwal V. and Sharma A.K., Microbial Pigments as Natural Colour Sources: Current Trends and Future Perspectives, *J Food Sci Tech.*, **52(8)**, 4669–4678 **(2015)**

89. Navin Raju V. and Radha T., Production of Extracellular Pigment from Microbes and Its Application, *Int. J. on Appl. Bioengineering*, **9(2)**, 23-29 **(2015)**

90. Valdes N., Soto P., Cottet L., Alarcon P., Gonzalez A., Castillo A., Corsini G. and Tello M., Draft genome sequence of *Janthinobacterium lividum* strain MTR reveals its mechanism of capnophilic behavior, *Std. in Genomic Sci.*, **24**, 110 **(2015)**

91. Venegas F.A., Köllisch G., Kerstin M., Diederich W.E., Kaufmann A., Bauer S., Chavarria M., Araya J.J. and García-Pineres A.G., The Bacterial Product Violacein Exerts an Immunostimulatory Effect Via TLR8, *Scientific Reports*, **9**, 13661 **(2019)**

92. Verinaud L., Lopes S.C.P., Naranjo Prado I.C., Thiago Alves da Costa F.Z., Gangi R.D., Issayama L.K., Carvalho A.C., Bonfanti A.P., Niederauer G.F., Duran N., Maranhao Costa F.T., Oliveira A.L.R., da Cruz Hofling M.A., Stach Machado D.R. and Thome R., Violacein Treatment Modulates Acute and Chronic Inflammation through the Suppression of Cytokine Production and Induction of Regulatory T Cells, *PLoS ONE*, **10(5)**, e0125409 **(2015)**

93. Wan A.A., Yusof N.Z., Nordin N., Zakaria Z.A. and Rezali M.F., Production and Characterization of Violacein by Locally Isolated *Chromobacterium violaceum* Grown in Agricultural Waste, *Appl. Biochem. and Biotech.*, **167(5)**, 1220-34 **(2012)**

94. Yang L.H., Xiong H., Lee O.O., Qi S.H. and Qian P.Y., Effect of Agitation on Violacein Production in *Pseudoalteromonas luteoviolacea* Isolated from a Marine Sponge, *Letters in Appl. Micro.*, **44**, 625-630 **(2007)**.

(Received 04th August 2021, accepted 10th October 2021)

*****