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Violacein: Review article

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Abstract :

The purple pigment violacein is produced by Gram-negative bacteria, mainly from the *Chromobacterium violaceum*. Violacein is synthesized by fusing two L-tryptophan molecules using five different enzymes encoded by *VioA, VioB, VioC, VioD*, and *VioE* genes. These genes have transferred to genetically engineering microorganisms such as *E.coli* for high production of violacein. It is receiving greater interest because of its significant biological functions and therapeutic potential. The reviews outlining the biosynthesis, production, and biological significance of violacein are being published.

Key words: Violacein, Deoxyviolacein, Oxyviolacein ,Purple pigment.

مقالة عن صبغة الفيولايسين

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مستخلص

يتم إنتاج صبغة الفيولايسين الأرجوانية بواسطة عدد من البكتيريا السالبة لصبغة الكرام، وبشكل رئيسي من بكتريا *Chromobacterium violaceum* ويتم تصنيع الفيولاسين عن طريق دمج جزيئين من الحامض الاميني التريبتوفان باستخدام خمسة إنزيمات مختلفة مشفرة بواسطة جينات *VioA , VioB , VioC , VioD* و *VioE* وقد تم نقل هذه الجينات إلى كائنات معدلة وراثيا مثل الإشريكية القولونية لزيادة انتاج الفيولايسين . صبغة الفيولايسين تحظى باهتمام كبير بسبب وظائفها البيولوجية الهامة وإمكاناتها العلاجية. لذلك هذا المقال سوف يوضح التركيب الكيميائي لصبغة الفيولايسين وكيفية انتاجها واهميتها البيولوجية.

الكلمات المفتاحية: الفيولايسين ، ديوكسي فيولايسين ، اوكسي فيولايسين ، الصبغة البنفسجية.

Introduction

Numerous distinct secondary metabolites are available in nature, such as pigments. Since the beginning of human history, pigments have been utilized for numerous purposes. They are still used now in numerous industrial sectors, including healthcare, textiles, meals, and cosmetics (Kulandaisamy *et al*., 2020). The essential pigment producers are microorganisms because they can be grown in regulated environments, require minimal nutrition, grow quickly and reproducibly, and are relatively easy to grow on a large scale. Moreover, extraction and purification of microbial pigments using well-established purification techniques that are commercially available. In addition, these pigments are environmentally friendly (Mumtaz *et al*., 2019).

A purple pigment violacein, known as 3-(1,2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol- 3-ilydene)-1,3-dihydro-2H-indol-2-one, (Duran *et al*., 2007) has recently caught the interest of scientists due to the wide range of biological activity it exhibits. In the past two decades, various papers have characterized violacein in terms of biological actions, such as antibacterial, immunomodulatory, antifungal , antiparasitic, antiviral, and anticancer (Duran *et al*.,2022).

Structure and properties of violacein

Violacein has three connected nitrogen-containing heterocycles in its chemical structure: a 5-hydroxylindole, an oxindole, and a 2-pyrrolidone (figure-1). Other derivatives that have been produced in addition to violacein are deoxyviolacein and oxyviolacein (Sarwar *et al*., 2021). Deoxyviolacein, compared to violacein, lacks an oxygen atom of the indole ring at position 6. At the same time, oxyviolacein has more oxygen atoms in the indole ring at position 20, as shown in figure-1. The violacein, which has a molar mass of 343.3 g/mol, exhibits substantial resonance-induced absorption in the spectrum of the visible region. It is water-insoluble but soluble in methanol, ethanol, and dimethyl sulfoxide. Due to its strong hydrophobicity (octanol-water partitioning coefficient log POW = 3.34), violacein is unlikely to be secreted into the environment by microorganisms(Duran *et al* ., 2021)

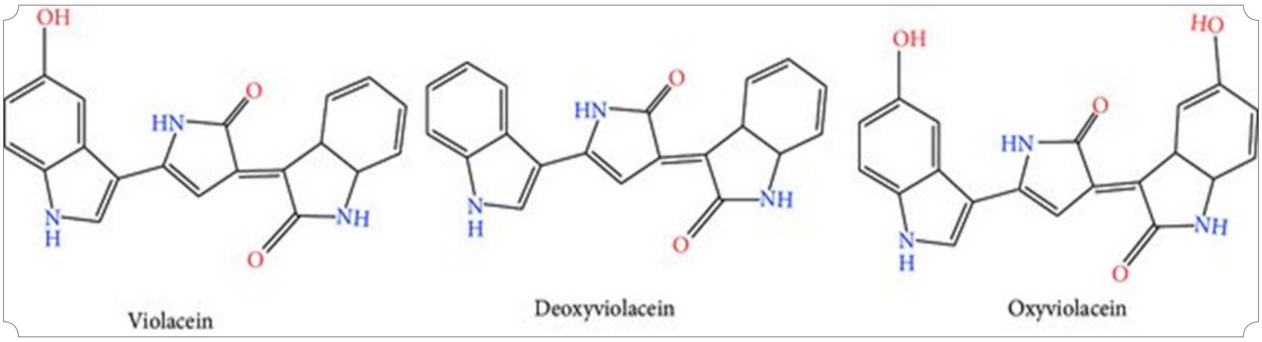

Figure-1: Structure of violacein, deoxyviolacein and oxyviolacein (Choi *et al* .,2015)

Violacein producing bacteria

Since it was first discovered more than 130 years ago, a number of violacein-producing bacteria have been identified. *Chromobacterium violaceum*, a species that has been widely studied as a model organism for the production of violacein (Choi *et al* ., 2015).A gram-negative *Chromobacterium violaceum*, inhabits soil and water, and visible violet colonies appear on typical media used in laboratories as a result of violacein producing by *C. violaceum*. When the stationary growth phase of *C. violacieum's* growth starts or when the nutrient supply decreases, violacein production is triggered. This phenomenon, which is referred to as quorum sensing (QS), occurs when a group of signaling molecules called N-Acyl homoserine lactones (AHLs) take part in a feedback loop to control gene expression (Ahmed *et al* ., 2021) .Numerous strains of *Janthino bacterium*, a species of gram-negative bacteria found in soil with various energy metabolism capabilities and endurance to cold, UV radiation, and other environmental stressors, have the ability to produce violacein. Other gram-negative bacteria like *Duganella* species *Alteromonas*, , *Pseudoalteromonas*, *Massilia* and *Collimonas* have the ability to produce violacein (Duran *et al* ., 2007). The violacein producers are diverse phylogenetically and also are places where they have been isolated, they involve a wide range of environments since these bacteria have been linked to the surfaces of marine sponges, the rhizosphere of olive groves, and even inside glaciers (Choi *et al* ., 2015).

Cloning and expression of the vioABCDE gene cluster on heterogeneous hosts, including *E. coli*, *Yarrowia lipolytica*, *Corynebacterium glutamicum*, and *Citrobacter freundii*, to solve the problems of low yields and pathogenicity among native producers (Ahmed *et al* ., 2021).

Violacein biosynthesis

Pemberton and his colleagues in 1991 first identified the violacein biosynthesis way, but it was completely described by Sanchez and his colleagues and Balibar his colleagues in 2006 (Tong *et al.*, 2021) .Violacein is synthesized by fusing two L-tryptophan molecules using five different enzymes encoded by *VioA, VioB, VioC, VioD*, and *VioE* genes, as shown in figure-2 .In addition to violacein, deoxyviolacein and oxyviolacein are also produced by this pathway.(Nemer *et al.*, 2023).First, the enzyme VioA (flavin-dependent tryptophan-2 monooxygenase) converts L-tryptophan into indole 3-pyruvic acid (IPA) imine. Secondly, IPA imine dimerised to a transitory imine dimer via VioB enzyme using heme b as a cofactor.Third, 1,2-shifting an indole ring by VioE, which transforms the imine dimer into protoviolaceinic acid (PDVA), without VioE, the unstable imine dimer transforms spontaneously into chromopyrrolic acid (CPA). Forth, the NADP-dependent oxygenase VioD then adds one hydroxyl group to one indole ring at position C5, converting PDVA into protoviolaceinic acid (PVA). Fifth, another NADP-dependent oxygenase, VioC, transforms PVA into violaceinic acid (VA) by linking a hydroxyl group to the C2 position of the other indole ring, then by the spontaneous oxidative decarboxylation that produces the ultimate byproduct, violacein. VioC promiscuity makes it possible for it to utilize PDVA as a substrate, which produces deoxyviolacein as the main byproduct, as shown in figure-2. Thus, in the presence of all five enzymes, “crude violacein” a combination of violacein and deoxyviolacein is produced, while the absence of vioD allows for the synthesis of pure deoxyviolacein. Oxyviolacein is produced by exogenous 5-hydroxy-l-tryptophan (5-HTP) since the enzymes involved in Vio biosynthesis lack rigorous molecular recognition and substrate specificity. Despite the fact that the same metabolic pathway enzymes are used for both the integration of 5-HTP into oxyviolacein and the conversion of l-tryptophan into Vio, as shown in figure-3 (Wang *et al* ., 2012).

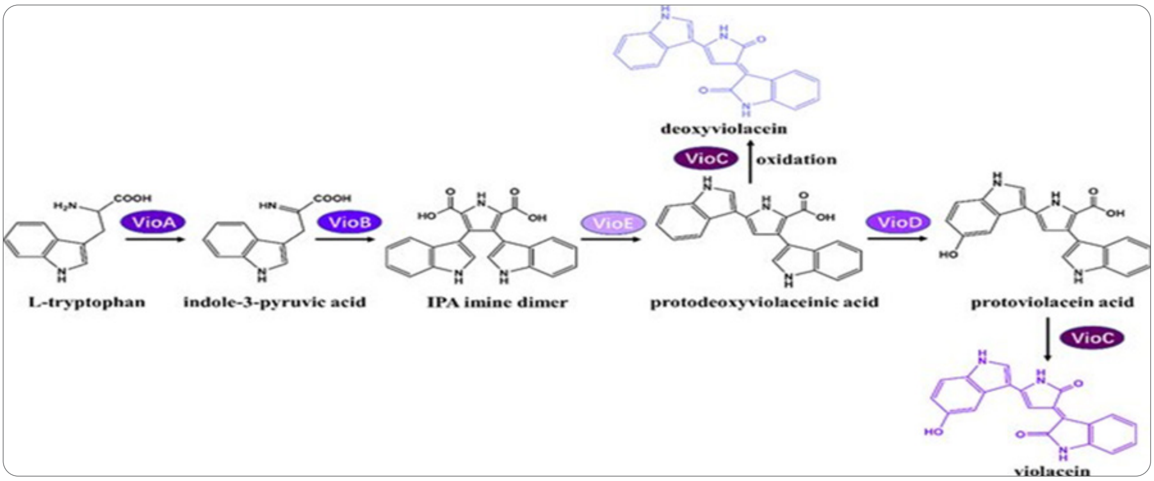

Figure-2: The violacein and deoxyvilacein biosynthetic pathway (Xu *et al*.,2022)

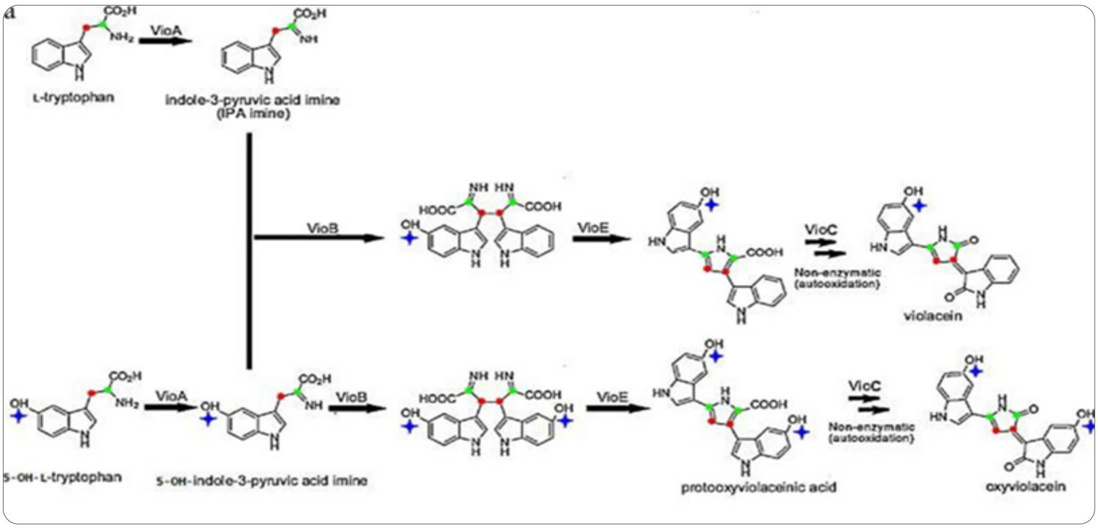

Figure-3: The violacein and oxyvilacein biosynthetic pathway (Wang *et al* ., 2012).

Biological activities of violacein

Violacein has established its commercial significance through a variety of industrial and biological processes like other pigments such as pyocyanine pigment (Mohammed *et al*.,2014, Oleiwi, 2015 , Qasim, 2019 and Abdali and Al-Attar,2020) and melanin pigment (Mahmood *et al*.,2016 and Gheni and Odaa,2023) which have numerous biological activities . Numerous studies have demonstrated that violacein possesses a variety of biological activities, among which are categorized as anti-fungal, antiviral, anti-bacterial, and anti-cancer (Ahmed *et al* ., 2021)

Antibacterial activity of violacein

Numerous investigations have been conducted to discover this violet pigment to clarify its antibacterial activities on various pathogens that might result in life-threatening infections in humans and other animals (Choi *et al* ., 2015).

Salmonella typhi, *Pseudomonas aeruginosa* , *S. aureus, Klebsiella pneumonia, S. epidermidis*, and *Vibrio cholerae* are just a few of the Gram-positive bacteria and Gram-negative bacteria that violacein exhibit exceptional antimicrobial activity on them. One key mechanism, it is assumed, connected to this action is the rupture of the cytoplasmic membrane (Duran *et al* ., 2007).

Cauz and his colleagues (2019) report that violacein directly destroys liposomes made up of synthetic and bacterial phospholipids, increasing their permeability, one of the key mechanisms, it is assumed, connected to this action is membrane rupture. Additionally, it was proposed that violacein-induced antibacterial action was the result of a two-step process (Aruldass *et al*. 2018). First, membrane permeabilization causes cellular leakage of proton, ion, ATP, and protein. finally, the osmotic imbalance caused by intracellular substance loss may result in cell death.(Duran *et al* ., 2007)

Violacein is being widely tested for its antibacterial action against *Staphylococcus aureus* both on its own and combined with other antimicrobial medicines and agents (Ahmed *et al* ., 2021). Remarkably, violacein has demonstrated a potent antibacterial action on *Staphylococcus aureus* isolated from bovine mastitis, a long-lasting condition linked with dairy farms that frequently exhibits multidrug resistance and causes large economic losses (Cazotto *et al*., 2011).

Along with having antibacterial properties, violacein has also demonstrated synergistic effects on mastitis when mixed with other antimicrobial agents. This result suggested using violacein in combination therapies (Duran *et al*., 2021)

The synergistic impact of violacein and commercial antibiotics has also been documented in several publications. Violacein coupled with other antibiotics, has been proven to have antibacterial efficacy against *S. epidermidis* (Dodou *et al*. 2017). Priya and his colleagues (2018) found that violacein has a synergistic interaction with commercial antibiotics that increases the efficacy of the drug.

Violacein was additionally investigated for its antibacterial capabilities combined with nanoparticles to improve their administration and for the synergistic antibacterial action. Both free violacein and violacein added into poly-(D,L-lactide-co-glycoside) nanoparticles were examined and demonstrated antibacterial activity on *S. aureus* ATCC 25923, when compared to the free violacein, the added violacein had three times the antibacterial action (Ahmed *et al* ., 2021)

Antifungul activity of violacein

Violacein also has activity on human fungal diseases and phytopathogenic fungi caused by various fungi species as mucormycosis, an opportunistic illness that can damage the skin and respiratory system and is caused by *Rhizopus arrhizus* and candidiasis (caused by *Candida auris*), a condition which can appear in a variety of ways, ranging from minor skin lesions and mucous membranes to a severe widespread form (Duran *et al*., 2022). Violacein also acts against *Rosellinia necatrix*, that causes a disease on mulberry known as white root rot (Duran *et al* ., 2007)

Antiviral activity of violacein

HeLa cells that had been post-infected with polioviruses and the Herpes Simplex Virus (HSV) were successfully treated with violacein . Its administration at a dosage of 0.25 g/ml decreased HSV by 62% while suppressing poliovirus-infected HeLa cells by 56%. These findings indicate that violacein has potent antiviral action (Duran *et al* ., 2022)

After infecting HeLa cells with the herpes simplex virus (HSV) and poliovirus, Andrighetti-Frohner and his colleagues (2003) used three distinct techniques to assess the cytotoxic and antiviral actions of violacein. At dosages which didn't impede cell growth, violacein didn't exhibit cytopathic or antiviral action on HSV-1 (29-R/acyclovir resistant strain), hepatitis A virus (strains HN175 and HAF-203) or adenovirus type 5.

According to Duran and his colleagues (2022), violacein may be employed as an immunotherapeutic drug for COVID-19 because it can function as a protease inhibitor at the level of the ACE2 receptor.

Industrial application of violacein

The textile industry utilizes the violacein pigment because of its characteristic purple color. It was produced by cultivating *Chromobacterium* and *Janthinobacterium* in a certain culture, and it was then utilized to color nylon fabric and fibrous materials. The same applies to synthetic (like nylon and vinylon) and natural (like silk, cotton, and wool) fibers (Duran *et al*.,2012)

In the formula of cosmetics, violacein and its derivatives are utilized like colors for coloring the skin and hair combined with a lipophilic and/or a hydrophilic material. Additionally, violacein-capped silver nanoparticles (vAgNPs) are used in the field of cosmetics for producing good skin items like anti-aging lotions (Arif *et al*.,2017).

Conclusion

This review discusses violacein, a purple secondary metabolite produced by different bacterial species that has shown promise in a number of biological and industrial applications . Violacein have used as anti-fungal, antiviral, anti-bacterial, and anti-cancer. In addition, its used as dyes in textile and cosmetic industry . Also, the violacein's structure, properties and biosynthetics have been discussed.

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