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Recent research advances on *Chromobacterium violaceum*

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ABSTRACT

Chromobacterium violaceum is a gram-negative bacterium, which has been used widely in microbiology labs involved in quorum sensing (QS) research. Among the QS-regulated traits of this bacterium, violacein production has received the maximum attention. Violacein production in this organism, however is not under sole control of QS machinery, and other QS-regulated traits of this bacterium also need to be investigated in better detail. Though not often involved in human infections, this bacterium is being viewed as an emerging pathogen. This review attempts to highlight the recent research advances on *C. violaceum*, with respect to violacein biosynthesis, development of various applications of this bacterium and its bioactive metabolite violacein, and its pathogenicity.

1. Introduction

Chromobacterium violaceum (*C. violaceum*) is a gram-negative beta-proteobacterium forming smooth violet colonies on common laboratory media. This violet color comes from the pigment violacein, encoded by the *vio* operon, whose expression is regulated by quorum sensing (QS) in this bacterium. Since this QS-regulated trait of *C. violaceum* is an easily observable and quantifiable marker trait, this bacterium has been widely used as a model organism in QS research labs. Though rarely implicated in human infections, infections caused by this bacterium are usually fatal. Traits other than violacein production in this organism have not been investigated that deeply. The famous violacein pigment produced by this bacterium is a bioactive molecule. Detailed investigations on the physiology and metabolism of this bacterium can further enhance its utility to biological research. Increasing interest of the research community towards *C. violaceum* is evident from the numerous papers being published on this bacterium (Figure 1). Using the search term '*C. violaceum*' in Google Scholar provides >11 000 results, of which nearly 3300 are post-2013. This article provides an overview of the recent research advances related to *C. violaceum*.

Most of the attention *C. violaceum* has attracted is owing to its bioactive pigment violacein, which is a secondary metabolite. Though not essential for growth and survival, violacein has been suggested to be a respiratory pigment, which also seems to be involved in regulation of tryptophan production [1]. *C. violaceum* is a facultative anaerobe, which tests positive for oxidase and catalase reactions. It grows optimally at 30–35 °C. It is a saprophyte found mainly in soil and water. Few details [2,3] regarding morphology, physiology, biochemical characteristics, antibiotic susceptibility/resistance of *C. violaceum* are presented in Tables 1–3. Complete genome of the *C. violaceum* has been sequenced. It consists of a single circular chromosome of 4 751 080 bp with an average G + C content of 64.83% [4]. Its pathogenic capability was first reported by Woolley in a fatal buffalo infection [5]. The first case of human infection was detected by Sneath *et al.* in 1927 in Malaysia [6]. In humans, it has been associated with infections of respiratory tract and gastrointestinal tract, liver abscesses [7], meningitis, endocarditis, hemophagocytic syndrome, and fulminant sepsis.

2. Violacein biosynthesis

C. violaceum produces a versatile blue-violet colored, water-insoluble pigment, violacein. Violacein production involves expression of *vio* operon consisting of five enzyme coding genes

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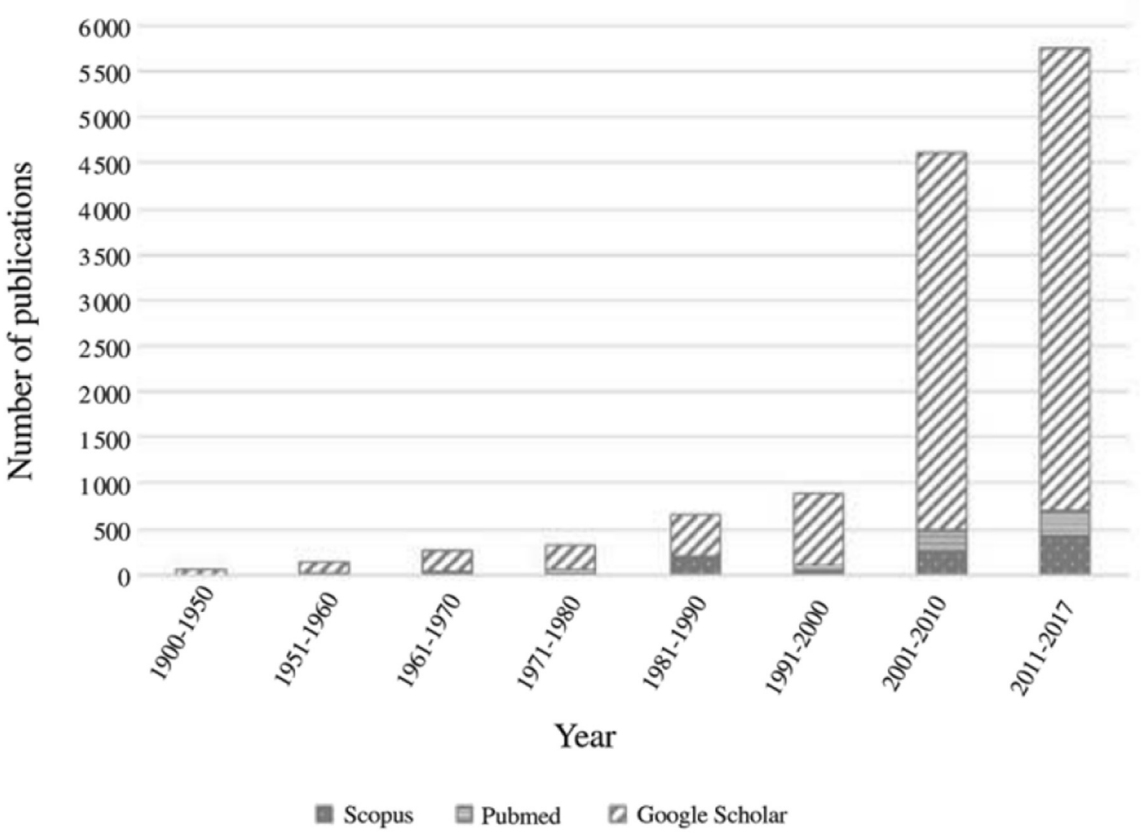

Figure 1. Increase in publications on *C. violaceum* over time.
For the year 2017, publications till March 2017 have been included.

Table 1
Morphological and physiological characteristics of *C. violaceum* [2,3].

No.	Characteristic	Description
1	Size	(0.6–0.9 × 1.5–3.0) μm
2	Shape	Rods with rounded end
3	Motility	Motile by means of a single polar flagellum
4	Growth at 4 °C	–
5	Growth at 30 °C	+
6	Growth at 37 °C	+
7	Growth at pH 4	+
8	Growth at pH 3	–
9	Growth in 1% NaCl	+
10	Growth in 2% NaCl	+
11	Growth in 4% NaCl	–
12	Anaerobic growth	+

'–' and '+' respectively indicates absence and presence of growth.

Table 2
Biochemical characteristics of *C. violaceum* [2,3].

No.	Characteristic	Description
1	Fermentation of glucose	+
2	Cyanide production	+
3	Lecithinase production (Turbid zone on egg yolk agar)	+
4	Acid production from	
	L-arabinose	–
	Trehalose	+
	D-cellobiose	–
	D-galactose	–
	Gluconate	+
	D-maltose	–
	N-acetyl glucosamine	+
5	Lactate utilization	+
6	Casein hydrolysis	+
7	Esculin hydrolysis	–
8	Arginine decarboxylase	+

'–' and '+' respectively indicates absence and presence of the respective metabolic activity.

(*vio*A, *vio*B, *vio*C, *vio*D, and *vio*E; Table 4) that are transcribed in one direction. These enzymes are coded by a 7.3 kb long DNA fragment [8]. QS system of *C. violaceum* consists of an evolutionarily conserved transcriptional regulator involving two adjacent genes, *cvi*I, an auto inducer synthase, and *cvi*R, a receptor [4] which regulates the production of violacein and other phenotypes in *C. violaceum*. Genes *cvi*I and *cvi*R are homologous to *Lux*I and *Lux*R respectively, of *Vibrio fischeri*. *cvi*I synthesizes auto inducer C6-homoserine lactone (HSLs) and *cvi*R codes for DNA-binding cytoplasmic transcription factor. These adjacent genes are transcribed from opposite DNA strands and express common regions of 73 bp [4].

QS is a cell-density related phenomenon. When cell density increases, AHL production also increases and it accumulates, which leads to the formation of a stable protein–ligand complex on binding with cytoplasmic CviR. Consequently AHL:CviR complex activates the transcription of *vio* operon by binding to its promoter site. On the other hand, when cell density is low, AHL concentration remains below the threshold, and formation of the signal–receptor complex does not take place. In this situation, the unbound CviR owing to its intrinsic instability, degrades rapidly resulting in no induction of QS-regulated genes. Using the recombinant *Escherichia coli* strains harboring mutations in *vio*A promoter, the palindromic DNA sequence 'CTGNCCNNNNGGNCAG' was identified as the binding site

Table 3
Susceptibility/resistance of *C. violaceum* to different antibiotics.

No.	Antibiotic	Concentration (μg/mL)	Interpretation
1	Ampicillin	25	Resistant
2	Chloramphenicol	10	Sensitive
3	Kanamycin	30	Sensitive
4	Neomycin	10	Sensitive
5	Streptomycin	10	Sensitive
6	Amikacin	30	Sensitive
7	Norfloxacin	10	Sensitive
8	Erythromycin	15	Sensitive
9	Amoxycillin	10	Intermediate
10	Co-trimoxazole	25	Sensitive
11	Ciprofloxacin	5	Sensitive
12	Netillin	10	Sensitive
13	Gentamicin	10	Sensitive
14	Cefadrozil	30	Resistant
15	Vancomycin	30	Intermediate
16	Ceftriaxone	30	Sensitive
17	Nitrofurantoin	300	Sensitive
18	Cloxacillin	1	Resistant
19	Penicillin	10 unit	Resistant
20	Tobramycin	10	Intermediate
21	Nalidixic acid	10	Intermediate
22	Ceftazidime	30	Intermediate
23	Cephoperazone	75	Intermediate

Information shown in this table has been sourced from Ref. [3], as well as from the antibiotic susceptibility tests executed in our lab with *C. violaceum* (MTCC 2656).

for CviR [9]. CviR has been shown to directly activate chitinase promoter, guanine deaminase genes, type VI secretion system, and positively regulate *cvi*I gene expression through a positive feedback system [8].

Biosynthesis of violacein (Figure 2) involves enzymatic oxidation of two molecules of L-tryptophan [10]. On the molecular level CviI synthase catalyzes conversion of fatty acids or S-adenosyl methionine into AHLs. This AHL forms a complex with CviR to trigger expression of the *vio* operon (*vio* ABCDE) unidirectionally. The first enzyme product, VioA (Flavin-dependent tryptophan 2-monooxygenase) catalyzes oxidation of tryptophan into indole-3-pyruvic acid imine (IPA) along with the reduction of FAD cofactor [8]. IPA is further converted into short-lived imine dimer through dimerization process, by the activity of VioB. The short-lived dimer is either spontaneously transformed into chromopyrrolic acid which is involved in indolocarbazole biosynthesis, or VioE modifies the dimer rapidly into protodeoxyviolaceinic acid (PVA) which leads to the biosynthesis of violacein. Thus, VioE can be said to play a key role in molecular skeleton construction of violacein [11]. Further, VioD yields proviolacein by catalyzing hydroxylation at the fifth position of one indole ring of PVA [12] along with the oxidation of NADPH to NADP. Proviolacein is then converted into violacein with the oxidation of NADPH into $NADP^+$ accompanied by formation of water and CO_2 under the control of VioC.

Different reports attach varying importance to different enzymes of violacein biosynthesis pathway. While VioE has been shown as the key enzyme by Hirano *et al.* as it can convert the short-lived dimer (IPA) rapidly into PVA that allows continuous violacein biosynthesis [11]; other reports [1,13] have shown VioB as the rate limiting enzyme. In our recent investigation [14] on violacein overproduction by sonic stimulated *C. violaceum*, *vio*B was found to be 3.92-fold up-regulated. In this case, increased violacein production seemed to have stemmed from higher expression of the genes participating in glucose metabolism through pentose phosphate pathway, resulting in availability of more erythrose-4-phosphate, to be used for tryptophan biosynthesis, which is the precursor for violacein synthesis. Employing a statistical approach for metabolic engineering, VioA and VioE were shown to be less important than VioB, VioC, and VioD during violacein production [13].

During violacein biosynthesis, byproducts oxyviolacein (having extra hydroxyl group) and deoxyviolacein (lacking hydroxyl group) are also produced. Till now, not much has been studied about these byproducts. Deoxyviolacein was shown to exhibit resistance to alkali/acid, and UV, and could inhibit proliferation of the hepatocellular carcinoma [15]. High production of deoxyviolacein was observed when *vio*D was knocked out from *vio* operon [16]. Absorption maxima of deoxyviolacein is at 560 nm [15], whereas the wavelengths used for violacein quantification are 575/580/585 nm. While extracting and quantifying violacein, it is important to avoid any possible interference from products like deoxyviolacein. Though *C. violaceum* has been used extensively in QS research, and violacein production has been given the maximum attention as its QS-regulated marker trait, not enough attention is always paid to avoid interference from violacein while quantifying bacterial growth photometrically. Violacein being a colored molecule, like any other pigment do absorb significantly at wavelengths conventionally used for measuring OD of bacterial cultures. We recently reported 764 nm as a better wavelength while performing photometric quantification of pigmented bacteria including *C. violaceum* [14]. Gallardo *et al.* reported utility of hyperspectral imaging for characterization of *C. violaceum* pigment at 580 nm and 764 nm [17].

3. QS-regulated traits of *C. violaceum*, other than pigment production

Though violacein production has received maximum attention as a QS-regulated trait in *C. violaceum*, other phenotypic characteristics (Table 5) like elastase production [18], and cyanide production [4,19], are also shown to be controlled via a

Table 4
Genes of the *vio* operon.

Gene	Gene symbol	Enzymatic action [8]	Molecular weight [8]	Length	PDB ID
*vio*A	CV_RS16140	Flavin-dependent tryptophan 2-mono-oxygenase	48	1257	5G3S
*vio*B	CV_RS16135	Considered to be a polyketide synthase, containing heme protein	111	2997	Not-available
*vio*C	CV_RS16130	FAD-dependent monooxygenase	48	1290	2WBO
*vio*D	CV_RS16125	Flavin-dependent monooxygenase	42	1122	3C4A
*vio*E	CV_RS16120	Responsible for conversion of flavanone to isoflavone	22	576	2ZF3

Base pair length for each of the gene was taken from Uniprot Databasee; Gene symbols were taken from: https://www.ncbi.nlm.nih.gov/gene; PDB ID were taken from Protein Data Bank (PDB).

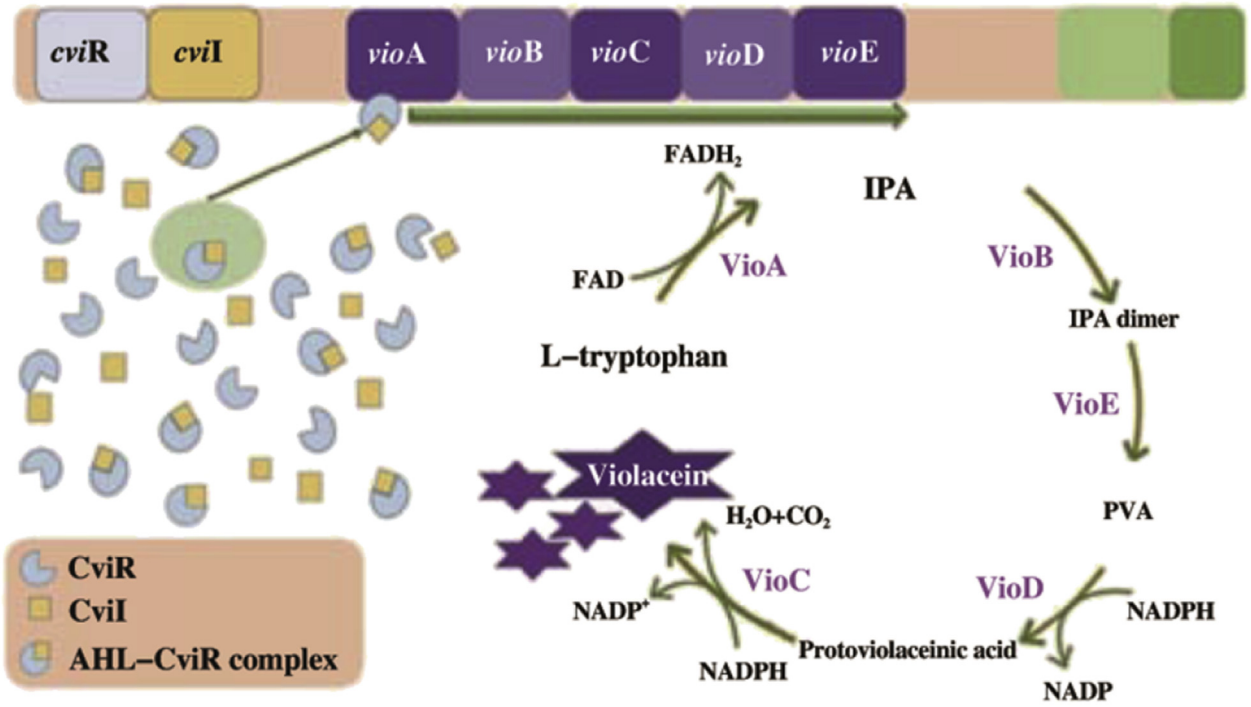

Figure 2. Schematic of violacein biosynthesis [8,9,11–13].
Binding of the CviR–AHL complex to the promoter site of *vioA* triggers expression of the *vio* operon, leading to synthesis of the purple pigment violacein.

Table 5
Various QS-regulated traits of *C. violaceum*.

QS-regulated trait	Gene(s)/Operon coding for the trait	Reference(s)
Violacein production	*vio* ABCDE operon (*vio*A, *vio*B, *vio*C, *vio*D, *vio*E)	[8]
Hydrogen cyanide production	*hcn*ABC operon	[4]
Cyanide degradation	*cyn*T (cyanate permease: CV1881) and *cyn*S (cyanase: CV1880)	[19]
Elastase production	*las*A and *las*B	[4,18]
Pilus	*pil*E2 (codes for type IV pilus protein)	[23]
Biofilm formation	*hms*HNFR	[20]
Chitinolytic activity	–	[24]

QS circuit mediated by the signal molecule, acyl homoserine lactone (AHL). Chitinase activity enables its survival on chitin as carbon source. Virulence of *C. violaceum*, like most other pathogens, is enhanced by its ability to form biofilm [20], and the biofilm mode also protects the organism from various stressful conditions. *C. violaceum*'s ability to cope with environmental stress is remarkable [4]. In our recent studies [21,22], when this bacterium was exposed to acoustic stress, it did respond by altering its growth and QS-regulated violacein production. In fact, some 342 genes were significantly differentially expressed in *C. violaceum* culture treated with sound corresponding to 300 Hz [14].

4. *C. violaceum* as a model for QS studies

Production of the easily detectable and quantifiable violacein pigment through a QS-regulated *vio* operon offers a simple way of screening for potential QS modulators and/or inhibitors. The ease of visualization and quantification of this QS-regulated pigment also opens the possibility of developing biosensors. *C. violaceum* CV026 is a biosensor strain employed widely for QS studies. It is a mini-T5-mutant of the wild type strain that lacks *cviI* encoded AHL synthase and thus can only produce violacein in response to externally supplied AHL signal molecules [25]. Such mutant strains can be useful for detecting presence of bacterial AHLs in any given environment. Strain CV026 was exploited for the identification of quorum sensing inhibitory (QSI) potential of Maniwamycins isolated from *Streptomyces* sp TOHO-M025 [26]. Lade and colleagues isolated a total of 200 bacterial strains that caused membrane biofouling in an activated sludge reactor by screening for AHL production using the biosensor systems *C. violaceum* CV026 and *Agrobacterium tumefaciens* A136 [27]. *C. violaceum* has proved very useful in assaying the anti-infective potential of many natural and synthetic products. Few examples of anti-QS activity detected from various sources, using *C. violaceum* as indicator organism are listed in Table 6. Quorum sensing modulators or quenchers can be applicable as potential alternatives to conventional bactericidal antibiotics. QS modulators may curb the virulence of the given pathogen without necessarily exerting a microbicidal action, and thus can be expected to pose lesser selection pressure on the pathogen population to develop resistance. *C. violaceum* was observed to show higher QS expression, when exposed to sub-inhibitory concentration of a broad range of antibiotics like gentamycin, tetracycline, erythromycin, amikacin, and kanamycin [32]. Besides being useful in the discovery of novel anti-infectives, *C. violaceum* is also a suitable organism for understanding the molecular details of QS phenomenon in gram-negative bacteria in general. Use of such model bacteria can help us understanding the mode of action of many of the traditional medicine, which is prescribed in ancient literature and being used in folk medicines since long with no precise insight into mechanism underlining their efficacy. Use of simple QS models like *C. violaceum* can help us in validating the antimicrobial claim of some of these

Table 6
Anti-Qs activity of few natural products against *C. violaceum*.

No.	Compound/extract	Source	Affected QS-regulated trait	Mode of action	Reference
1	Secondary metabolites	*Halobacillus salinus*	Violacein biosynthesis	Antagonizes bacterial quorum sensing by competing with N-acyl homoserine lactones for receptor binding	[28]
2	Maniwamycins	*Actinomycete* strain TOHO-M025	Violacein synthesis	–	[26]
3	Carvacrol	Oregano oil	Biofilm formation, violacein production and chitinase activity	*cvi*I gene expression is reduced	[29]
4	Guava leaf aqueous extract	*Psidium guajava*	Violacein production	–	[30]
5	Alcoholic extract of plant leave	*Syzygium cumini*, and *Pimenta dioica*	Violacein production	–	[31]

traditional medicinal practices e.g. use of pomegranate peel or coffee as antimicrobial substances. Pomegranate peel and the coffee constituent-caffeine have been shown to possess QS inhibitory potential [33,34]. We recently demonstrated the mode of action of an anti-infective polyherbal ayurvedic formulation (Panchvalkal) to be based on its quorum-modulatory potential, wherein *C. violaceum* was one of the test strains [35].

5. Pharmaceutical and commercial importance of violacein

Besides being a marker molecule for QS studies, violacein has also received considerable attention owing to a variety of biological activities possessed by it. While on one hand, those working in the area of anti-infectives look for inhibition of QS-regulated violacein production in *C. violaceum*, those focusing on violacein as a bioactive molecule look for improving the fermentative yield of violacein [13,16]. Violacein has been reported for its anti-proliferative activity against cancer cells lines [36], antibacterial activity against gram-positive bacteria like multidrug resistant *Staphylococcus aureus* (*S. aureus*) [36,37], antifungal activity [38], trypanocidal and anti-leishmanial property [39], *etc*. Leishmaniasis affects 12 million people worldwide and is sometimes fatal. Anti-leishmanial activity of violacein at 10 μmol/L for 7 consecutive days was observed in male albino mice with no side effects [40].

Though violacein production in *C. violaceum* has received much attention, there are other organisms too, in whom violacein production has been reported, such as *Duganella violaceinigra* str. NI28 [16], *Pseudoalteromonas luteoviolacea* [41], *Janthinobacterium lividum* [42], *etc*. Violacein exhibited anticancer activity against human MCF-7 breast cancer cells in a time and dose-dependent manner, with IC_{50} values of 4.5 μM in 24 h, 1.7 μM in 48 h, and 0.51 μM in 72 h by generating significant ROS production even at lower doses [43]. Inhibitory effect of violacein on *S. aureus* growth at concentrations between 5.7 and 15 mg/L (approximately 17–43 mol/L) has been reported [44]. Use of violacein in combination with other antimicrobial agents was indicated by Subramaniam *et al.* based on minimum inhibitory concentration (MIC) data for violacein, violacein–gentamicin, violacein–cefadroxil, and violacein–kanamycin combinations. Violacein–gentamicin and violacein–cefadroxil combinations registered appreciably low MIC of 1.0 μg/mL against *S. aureus*. Most violacein–macrolide and violacein–aminoglycoside class combinations revealed fractional inhibitory concentration indices (FICI) of <0.5, indicating synergism. Similarly, violacein–azithromycin and violacein–kanamycin combination, exhibited significant synergy (FICI: 0.3) against *Salmonella typhi*. Violacein alone registered MIC value of 5.7 μg/mL (*S. aureus*), 15.6 μg/mL (*Klebsiella pneumoniae*), 18.5 μg/mL (*Pseudomonas aeruginosa*), 20.0 μg/mL (*Vibrio cholerae*) and 5.7 μg/mL (*S. typhi*) [45].

Anti-protozoal activity of violacein was studied against various protozoan species of flagellates, ciliates, and amoeba, and also against the bacterivorous nematode worm *Caenorhabditis elegans* (C. *elegans*), which is a premier genetic model organism. *C. elegans* grows healthily in the laboratory when normally fed with auxotrophic *E. coli* as a prey. However, when fed with two violacein producing bacterial strains *Janthinobacterium* sp. HH01 and *C. violaceum*, severe toxic effects were observed, affirming that violacein is toxic to the bacterial predator *C. elegans* [46]. Violacein has also been shown to possess strong antioxidant potential, and could increase the sun protection factor (SPF) of commercial sunscreens and thus present new useful paradigm for sunscreens to be manufactured by utilizing substances of biological origin [47]. Violacein has also been employed as a robust bacterial label for non-invasive optoacoustic imaging with high potential for basic research and future theranostic applications in bacterial tumor targeting [48]. Potential use of violacein as an antifungal agent especially against dermatophytic fungi *Trichophyton rubrum*, a major human pathogen has also been indicated [49]. A team led by Stefanie Lopes showed that micro molar concentrations of violacein efficiently killed chloroquine-sensitive and -resistant *Plasmodium falciparum* strains *in vitro*, inhibited parasitemia *in vivo* even after parasite establishment, and protected *Plasmodium chabaudi chabaudi*-infected mice from a lethal challenge [39].

6. *C. violaceum* as an emerging pathogen

Though *C. violaceum* is primarily considered to be an environmental organism, not regularly associated with human infections, sporadic cases on its involvement in human infections have been reported, and mortality rate in such cases is found to be high. *C. violaceum* infection is reported to be fatal, whenever it occurs in human subjects, and it is being considered as an emergent pathogen [50–53]. Treatment of *C. violaceum* infections can prove challenging owing to its resistance against different antibiotics. It is known to be resistant to penicillins and cephalosporins [54,55]. Cases of *C. violaceum* infection in

humans have been reported from Australia, India, Nepal, USA, Brazil, and Congo [56–64]. Age of patients indicated in these case reports ranges from 11 months to 73 years. Specimens from these patients used for diagnosis include blood, urine, pus sample, and scrapings from the oropharynx. Out of 11 cases listed at number [52,53,56–64], 5 of the patients could not be treated successfully and died, which indicates the severity of *C. violaceum* infection. All these cases are post-2013, suggesting the emerging status of this bacterium as a pathogen. Few representative cases are described below, for details of the individual case report, readers may refer the above-cited references.

In the case of a 45 years old male patient from Nepal mentioned in the study of Ansari *et al.* [61], the patient was suffering from fever and breathing difficulty for two days. Examination of the pus sample from his prick injury in finger revealed the infection to be caused by *C. violaceum*. Though four different antibiotics (piperacillin, tazobactam, flucloxacillin, and metronidazole) were employed for treatment, this patient could not be saved from death. In another case of a 30 years old male from Congo, mentioned in the study of Bottieau *et al.* [53], the patient was suffering from fever, cough, thoracic pain, and vomiting. Examination of his blood specimen revealed presence of *C. violaceum*. Despite ceftriaxome and gentamicin treatment, this patient succumbed to death. Similar example of a 10 years old Indian boy has been mentioned in the study of Kar *et al.* [64], who was suffering from acute abdominal pain, high fever, and vomiting, ultimately died of renal and cardiorespiratory failure, despite amikacin and metronidazole administration. On the other hand, an Indian male aged 73, despite being old (and hence may not be at the peak of his immunity) could recover successfully from and urinary tract *C. violaceum* infection, following a levofloxacin treatment [57]. An overview of different case studies, where the patient could recover, seems to suggest that ciprofloxacin is one of the antibiotics effective against *C. violaceum* [52–62].

Thus, *C. violaceum* infection can be said to be difficult to treat. Mortality rate can be said to be high, and successful treatment usually involves use of multiple antibiotics. Infections by this bacterium though not large in number, sporadic cases have been reported from a wide range of geographical locations. It is advisable for the researchers working with this bacterium to practice appropriate safety precautions.

7. Patents related to *C. violaceum*

Interest of the research community in developing various applications of *C. violaceum* is evident from many patents filed, mentioning this bacterium. Using the search term '*C. violaceum*' yielded 40 patents in European Patent Office (EPO) website (https://www.epo.org/index.html), whereas three patents appeared using the search term '*C. violaceum*' in Indian patent database (https://ipindiaonline.gov.in/patentsearch/search/index.aspx). These patents exemplifies that *C. violaceum* can have diverse applications in areas like bioleaching, pigment production, biosensors etc, in addition to its most common use as an assay strain for novel QS modulators [65–77]. However, its ability to act as a pathogen is a factor to be considered seriously while developing any such applications. Owing to this fact, violacein production from alternative sources has also been researched [73].

Haifeng and Lei patented a method for purification of romidepsin from *C. violaceum* broth [68]. This method was claimed to offer advantages of high extraction purity, high yield, simplicity, and low cost. Another patented method for dyeing polyester fabrics using colorant extracted from *C. violaceum* is a good example of *C. violaceum* application in textile industry [69]. In one patent [71], a newly reported strain of *C. violaceum i.e. Chromobacterium* sp. HS-13-94 was claimed to generate disulfide-bond depsipeptide type compound as an active substance, which exerts inhibitory effect against tumor cells of human body, and displays a wide anti-tumor spectrum. Kleid *et al.* patented a process for recovery of gold from its ore, using *C. violaceum* and *Chlorella vuloaris* [72]. de Vasconcelos *et al.* were granted a patent regarding applications of *C. violaceum* polynucleotides expressed as a part of stress response in this bacterium [74]. A large fraction of patents [66,67,70] mentioning *C. violaceum* describe this bacterium as a suitable test organism which can be challenged with potential QS inhibitors during *in vitro* and *in vivo* experiments. *In vivo* assays have most commonly employed the nematode worm *C. elegans* as the model host, which can easily be infected by *C. violaceum*. We do not intend to do an extensive review of *C. violaceum* patents here. The patents mentioned in this review are just to give the reader an idea about the type of applications involving *C. violaceum* being patented, and the potential of commercialization. Few patents [75–77] with the status 'application under examination' have also been mentioned.

8. Concluding remarks

C. violaceum can be considered to be a bacterium famous in the research community. The single largest factor contributing to the research community's attention to this bacterium is presence of the purple pigment violacein, which is a very useful marker trait during QS assays. Violacein itself has been a molecule of sufficient interest owing a large variety of biological activities reported in it. Though alteration in violacein producing capacity of *C. violaceum* is often associated with activity of its QS circuit, it should be noted that violacein production in this bacterium is not exclusively controlled by the QS machinery. Bearing this fact in mind is essential to accurate interpretation of assay relying largely on quantification of violacein as a measure of QS activity. In our study [14] of sonic stimulated *C. violaceum* culture, in response to the acoustic stress cell yield was found to increase up to a minor extent only, whereas violacein production was enhanced at a much higher magnitude. This suggests that violacein production is not that tightly associated with (or regulated by) cell density. QS signals may not be the sole control strategy in *C. violaceum* for violacein production. Even in densely populated cultures, grown in high oxygen and glucose levels, violacein production can be suppressed [1]. Violacein production can also be considered to be dependent on the type and concentration of the carbon source in the growth medium, pointing to the possibility of a control mechanism similar to that mediated by cyclic AMP. Recently Devescovi *et al.* have reported that the biosynthesis of violacein is negatively controlled by a novel repressor protein, VioS [78]. The violacein operon is regulated negatively by VioS. Latter does not regulate the CviI/R system. Apart from violacein, VioS, and QS regulate other

phenotypes in an antagonistic fashion. QS-regulated phenotypes in *C. violaceum* thus seem to be further regulated providing an additional level of control.

Another important point to be considered while assaying different test products for their potential anti-QS activity against *C. violaceum* is that many of these test substances are dissolved in solvents like DMSO, which itself can affect QS-regulated violacein production in this bacterium [79], and/or can potentiate/mask the effect of the test formulation. Hence, it is necessary to have proper independent investigations in different labs regarding how DMSO affects violacein production, and what may be considered as the optimum concentration of DMSO to be allowed in such assay systems. Including the vehicle control in such experiments cannot tell us about the possible potentiating effect of DMSO on test formulations.

Future research on *C. violaceum* can expand its utility to areas of research other than QS too, such as its use as a model bacterium for studying bacterial stress response, in bioleaching, bioremediation, biosensors, *etc*. We can expect many interesting aspects of the biology of this colorful bacterium to get unfolded in times to come, which not only will help in developing new applications, but also in better managing *C. violaceum* infections in humans and animals.

Conflict of interest statement

We declare that we have no conflict of interest.

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