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Research review paper

Advances in *Chromobacterium violaceum* and properties of violacein-Its main secondary metabolite: A review

Nelson Durán [a,b,c,*], Giselle Z. Justo [d], Marcela Durán [b,e], Marcelo Brocchi [f], Livia Cordi [b,f], Ljubica Tasic [a,b], Guillermo R. Castro [g], Gerson Nakazato [h]

[a] Institute of Chemistry, Biological Chemistry Laboratory, University of Campinas, CP 6154, CEP 13083-970 Campinas, SP, Brazil
[b] NanoBioss, Institute of Chemistry, University of Campinas, Campinas, SP, Brazil
[c] LNNano (CNPEM) Campinas, SP, Brazil
[d] Department of Cell Biology and Department of Biochemistry, Federal University of São Paulo (UNIFESP-Diadema), SP, Brazil
[e] Institute of Biology, Urogenital, Carcinogenesis and Immunotherapy Laboratory, University of Campinas, SP, Brazil
[f] Institute of Biology, Department Genetics, Evolution and Bioagents, Universidade Estadual de Campinas, Campinas, SP, Brazil
[g] Nanobiomaterials Laboratory, Applied Biotechnology Institute (CINDEFI, UNLP-CONICET CCT La Plata) – School of Sciences, Universidad Nacional de La Plata, La Plata, Argentina
[h] Department of Microbiology, Biology Sciences Center, Londrina State University (UEL), Londrina, Brazil

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ABSTRACT

Chromobacterium violaceum is important in the production of violacein, like other bacteria, such as *Alteromonas*, *Janthinobacterium*, *Pseudoalteromonas*, *Duganella*, *Collimonas* and *Escherichia*. Violacein is a versatile pigment, where it exhibits several biological activities, and every year, it shows increasing commercially interesting uses, especially for industrial applications in cosmetics, medicines and fabrics. This review on violacein focuses mainly on the last five years of research regarding this target compound and describes production and importance of quorum sensing in *C. violaceum*, mechanistic aspects of its biosynthesis, monitoring processes, genetic perspectives, pathogenic effects, antiparasitic and antimicrobial activities, immunomodulatory potential and uses, antitumor potential and industrial applications.

Contents

* Corresponding author at: Biological Chemistry Laboratory, Institute of Chemistry, University of Campinas, CP 6154, Campinas, SP CEP 13083-970, Brazil.
E-mail address: duran@iqm.unicamp.br (N. Durán).

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1. Introduction

Chromobacterium violaceum has been extensively studied in the violacein production field, even though different yields and production conditions have been published for other bacterial strains (Durán and Menck, 2001; Durán et al., 2007, 2010, 2012; Choi et al., 2015a,b).

Violacein producers vary phylogenetically, and so the production of this pigment depends on the location where the bacteria have been isolated. Table 1 includes a selection of environments and different countries where violacen production had been reported. Perhaps the best known genus is *Chromobacterium* (Moss et al., 1978; Hoshino, 2011), which includes the species *C. violaceum* (Durán and Menck, 2001).

Table 1
Violacein production from various microorganisms.

Strains	Comments	References
Alteromonas luteoviolacea	Marine bactéria, Scotland	Laatsch and Thomson (1984)
Chromobacterium violaceum	Bacillus violaceum, USA	Tobie (1934)
Chromobacterium violaceum ATCC 553	Collection	De Moss and Happel (1959)
Chromobacterium violaceum	Lowland river, England	Moss et al. (1978)
Chromobacterium violaceum B78	From Amazon River, Manaus, Brazil	Riveros et al. (1989)
Chromobacterium violaceum CCT 3496	Collection	Rettori and Durán (1997), Rettori et al. (1998)
Chromobacterium violaceum CCT 3496	Collection	Mendes et al. (2001a, b)
Chromobacterium violaceum	Agricultural waste, Malaysia	Ahmad et al. (2012).
Chromobacterium violaceum (MTCC 2656)	Indian Collection	Chaudhari et al. (2014)
Citrobacter freundii/ pCOM10vio	Recombinant strains	Jiang et al. (2010), Yang et al. (2011)
Collimonas sp.	Costal water, Norway	Hakvag et al. (2009)
Duganella violaceinigra	Forest soil	Li et al. (2004)
Duganella violaceinigra str. NI28	Forest soil	Choi et al., 2015b
Duganella sp. B2	From China	Wang et al. (2009)
Duganella sp.	Agricultural soils (olive), Spain	Aranda et al. (2011)
Escherichia coli K12 DH5a	*E. coli* K12 cloning	Ahmetagic and Pemberton (2010)
Escherichia coli MG1655-Vio4	Recombinant strains	Rodrigues et al. (2013)
Escherichia coli BL21(DE3)/ pET32avio	Recombinant strains	Jiang et al. (2010)
Escherichia coli Bl21(DE3) B2/pED + pVio	Recombinant strains	Fang et al. (2015)
Janthinobacterium lividum S9601	Collection	Shirata et al. (1998)
Janthinobacterium lividum strain DSM1522	Collection	Pantanella et al. (2007)
Janthinobacterium lividum	Glacier, China	Lu et al. (2009)
Janthinobacterium svalbardensis	Glacier, Slovenia	Avgustin et al. (2013)
Pseudoalteromonas DSM 13623	Marine sediment bacterium Germany Patent	Tan et al. (2002)
Pseudoalteromonas sp.	Deep sea Waters, Japan	Yada et al. (2008)
Pseudoalteromonas luteoviolacea	Marine sponge, China	Yang et al. (2007)
Psychotropic bacterium RT102	Close to *J. lividum*	Nakamura et al. (2002, 2003)
Psychrotrophic bacterium, XT1	Close to *J. lividum*	Lu et al. (2009)
Pseudoalteromonas sp. 520P1	Pacific coast. Japan.	Dang et al. (2014)

Most recently, there have been different strategies for studying violacein producers in different parts of the world. Aranda et al. (2011), using different aspects such as microbiological, physiological and genetic analyses, isolated and identified *Duganella* spp. that was associated with the rhizosphere (plants) produced violacein. Seven isolated *Duganella* spp. strains produced high levels of violacein in vitro, contrary to previously published reports. Violacein showed growth-inhibitory activity against Gram-positive bacteria but not against Gram-negative bacteria and fungi. Different *Duganella violaceinigra* from Forest soil were isolated and characterized and showed excellent productivity of violacein (Li et al., 2004, Choi et al., 2015b). Ahmad et al. (2012) isolated *C. violaceum* from various plant waste sources, such as bagasse, fruit waste, molasses, and others, as an alternative to the rich medium normally used. Among them, sugar bagasse supplemented with L-tryptophan was the most efficient in the production of violacein when compared with common nutrients.

Janthinobacterium svalbardensis (JA-1 strain) was isolated from Norway glacier ice samples (Avgustin et al., 2013). The 16S rRNA gene sequences and DNA–DNA hybridization tests demonstrated that the JA-1 strain, although belonging to the genus *Janthinobacterium*, represented a novel lineage distinct from the two known species of this genus, *J. lividum* and *J. agaricidamnosum*. The isolate was a psychrotrophic Gram-negative bacterium, which, as rod-shaped with rounded ends, contained intracellular inclusions and one polar flagellum. On the basis of the results, authors proposed that strain JA-1 was the type strain of a novel species of *Janthinobacterium*, for which the name *Janthinobacterium svalbardensis* sp. nov. was given.

In the work of Rodrigues et al. (2013), systems-wide metabolic engineering was used to target *Escherichia coli*. The basic producer, *E. coli* dVio-1, that expressed the vioABCE cluster from *C. violaceum* under control of the inducible *araC* system, accumulated deoxyviolacein. Through intracellular metabolite analysis, bottlenecks in tryptophan supporting pathways were identified, which is the major product building block. This was used for understanding the engineering of serine, chorismate and tryptophan biosynthesis and the pathways of the non-oxidative pentose-phosphate process. The ultimate strain, *E. coli* dVio-6, accumulated deoxyviolacein in shake flask cultures. The created system of a high-flux tryptophan pathway was fulfilled by genomic integration of the vioD gene of *J. lividum* (exclusive production of violacein). Finally, in a fed-batch process, *E. coli* Vio-4 accumulated the violacein as the main product.

Chaudhari et al. (2014) showed that dimethyl sulfoxide (DMSO) enhanced violacein production in *C. violaceum* (MTCC 2656) in a dose-dependent manner, simultaneously exerting an inhibitory effect on bacterial growth. Thus, the effect of DMSO on violacein production by the cells was increased due to interference with the quorum sensing (QS) of *C. violaceum*.

It was known that in *Vibrio fischeri*, there are essential components in QS-regulated bioluminescence, such as N-acylhomoserine lactone (AHL) synthase (LuxI) and AHL receptor protein (LuxR), and their genes (*luxI*/*luxR*) have been determined (Lazdunski et al., 2004). The identification of these genes led to the understanding of the mechanisms of QS regulation and the nature of AHLs related to violacein production. Dang et al. (2014) sequenced the complete genome of *Pseudoalteromonas* strain 520P1 no. 412 (NBRC 107704) identifying the *luxI* and *luxR* genes. These authors reported a draft 5.25-Mb genome sequence of *Pseudoalteromonas* sp. 520P1 (marine violacein-producing bacterium) from the Pacific coast of Japan. BLAST searches (genome annotation) demonstrated the presence of one *luxI* and five *luxR* homologs. Subsequently, there was an important comparative study of the genomes with two other known violacein-producing *Pseudoalteromonas* (Thomas et al., 2008; Cress et al., 2013), which assisted to determine the protein components involved in the regulation of quorum-sensing in violacein synthesis.

An interesting strategy was followed by Fang et al. (2015) to enhance violacein production. Strains with a multivariate module for