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Action and function of *Chromobacterium violaceum* in health and disease: Violacein as a promising metabolite to counteract gastroenterological diseases

Giselle Zenker Justo [a, *], Nelson Durán [b, c, d]

[a] Department of Pharmaceutical Sciences and Department of Biochemistry, Universidade Federal de São Paulo (UNIFESP), 3 de Maio, 100, 04044-020, São Paulo, SP, Brazil
[b] Biological Chemistry Laboratory, Institute of Chemistry, Universidade Estadual de Campinas, CP 6154, 13083-970, Campinas, SP, Brazil
[c] NanoBioss, Institute of Chemistry, Universidade Estadual de Campinas, Campinas, SP, Brazil
[d] LNNano (CNPEM), Campinas, SP, Brazil

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ABSTRACT

Chromobacterium violaceum is a Gram negative, β-proteobacterium found in the microbiota of tropical and subtropical environments. Although considered an opportunistic pathogen, infection rapidly progress to fatal sepsis, with metastatic abscesses. It is noteworthy the multidrug resistant phenotype of *C. violaceum* and the possibility of relapse. Recently, an influence of global climate in the incidence of cases beyond the previous areas has been observed. Furthermore, chronic granulomatous disease has been considered a risk factor to infection. Despite the increase in *C. violaceum* infection incidence and high mortality, most clinicians are not familiar with it. This review pointed out important features of this life threatening microorganism, including its pathogenicity, mechanistic aspects, genetic and drug resistance associated factors, and the clinical association with chronic granulomatous disease. In addition, its main metabolite violacein may be a promising agent to counteract gastroenterological diseases, such as colorectal cancer and inflammatory gastric lesions.

Introduction

Secondary metabolites have a privileged place in microbiology since the discovery of penicillin by Fleming, which pave the way for the identification of a valuable number of medicinal compounds. It is therefore expected that tropical regions may contribute with a biodiversity with potential biotechnological and pharmaceutical applications [1]. In fact, nature still is an inexhaustible source of microbial diversity awaiting exploration, and *Chromobacterium violaceum* is one of its treasures described more than a century ago, together with its main metabolite violacein, identified as responsible for the violet colour of the bacteria colonies. In 1976, *C. violaceum* was first isolated in the borders and water of the Negro river (Amazon, Brazil), leading to the characterization of the photobiological properties of violacein, [3-(1,2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1,3-dihydro-2H-indol-2-one]. As a consequence of this preliminary studies and its high abundance in the Amazon region, this bacterium and its main metabolite have been studied in Brazil for the last four decades and more recently it has attracted interest from the scientific community worldwide [2–5].

Chromobacterium violaceum is a Gram-negative, rod-shaped, motile, non-fastidious, non-sporing, facultatively anaerobic, fermentative and positive for catalase and oxidase bacterium, widely distributed in the microbiota of tropical and subtropical regions. When incubated in nutrient agar, blood agar or MacConkey agar media, it produces colonies with a dark purplish colour in the pigmented strain due to its metabolite, violacein [6,7]. However, pigment production cannot be considered a trait of pathogenicity, since nonpigmented strains may have similar virulence [8–11]. Microorganism identification depends on the biochemical characterization, although detection using multiplex polymerase chain reaction targeting the prgI, spaO, invG, and sipB genes and sequencing of the 16S rDNA gene have been demonstrated [12,13].

Although this microorganism is not damaging to plants, and is

* Corresponding author. Department of Biochemistry, Universidade Federal de São Paulo, 3 de Maio, 100, 04044-020, São Paulo, SP, Brazil.
E-mail addresses: giselle.zenker@unifesp.br (G.Z. Justo), duran@iqm.unicamp.br (N. Durán).

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only opportunistic to animals and humans, the infection may rapidly progress to life threatening sepsis, representing a difficult-to-treat entity. Moreover, there is a rise in the number of patients who presented with *C. violaceum* infection beyond the bacterium previous tropical and subtropical ecosystems [5,7,14]. This is of most importance since the geographic distribution of the bacterium may follow changes in global warming [7].

It is worth noting that genetic studies of *C. violaceum*, mainly conducted by the Brazilian National Genome Sequencing Consortium using the ATCC 12472 strain in 2003, revealed important characteristics of the microorganism, supporting its adaptability to the environment. In addition, these studies also provided information about the pathogenicity, metabolism, host interaction and violacein biosynthesis [15]. Accordingly, this strain was demonstrated to contain a circular chromosome of 4,751,080 bp and a G + C content of around 64,83%. Interestingly, these studies also revealed that several mechanisms contribute to *C. violaceum* coping with the plethora of stressors present in Negro river environment, such as high temperatures, lack of nutrients, high levels of radiation and toxic agents, thus justifying its abundance in this region [15]. In addition, the molecular mechanisms associated with its pathogenicity were proposed on the basis of the analysis of genes encoding possible virulence factors [15]. An important finding of this study was the presence of ORFs enconding type III secretory systems (T3SS or TTSS). T3SS is encoded by the *Chromobacterium* pathogenicity islands 1 and 1a (Cpi-1/-1a), and is involved in the translocation of proteins into the cell cytosol, where they take advantage of cellular signal transduction cascades for the pathogen benefit [16,17]. The Cpi-1/-1a-encoded T3SS is a chief inducer of cell death in a murine model of *C. violaceum* infection and in cultured mammalian cell lines, through the formation of pore structures in the host cell membrane [18]. Recently, these same authors demonstrated that CilA is a major regulator of the T3SS and they further characterized an effector protein translocated by this system, CopE (*Chromobacterium* outer protein E), which is a guanine nucleotide exchange factor (GEF) for Rac1 and Cdc42, thus playing a key role in bacterial infection of epithelial cells [17]. In addition, genes encoding factors associated with adherence and invasion process, synthesis of lipopolysaccharide (LPS) and peptidoglycan, and cytolytic proteins, such as hemolysin-like proteins, were reported [15]. Recent studies using mass spectrometry demonstrated the presence of hemolysin, collagenase, flagellar protein, metallopeptidases, outer membrane proteins, as well as the type IV secretory system (T4SS) effector protein in the culture medium of *C. violaceum* [19,20].

Pathogenicity of Chromobacterium violaceum

Despite the ample distribution of *C. violaceum* in Negro river, which is a source of drinking water for the population, this saprophyte bacterium rarely infects humans, with most cases occurring in immunocompromised individuals or children [21]. This was suggested to be related to the lack of some invasion systems currently found in other proteobacteria, such as *Salmonella typhimurium* and *Yersinia pestis* [15]. However, infections have been reported to cause skin lesions, meningitis, endocarditis, localized or metastatic abscesses, osteomyelitis, hemophagocytic syndrome, peritonitis, respiratory distress syndrome, gastrointestinal infection and sepsis, with a fatal outcome in several cases. Indeed, a rapid progression to fulminant sepsis and multiple organ dysfunction is commonly observed in *C. violaceum* infection. Urinary tract infection and diarrhea may also occur [5,7]. There is no age or gender preference and chronic granulomatous disease and glucose 6-phosphate dehydrogenase deficiency are the only conditions that seem to predispose to *C. violaceum* infection [22,23].

Other important features of the infection are its multidrug resistance and the possibility of relapse [7,25]. High mortality rates varying from 53 to 80% have been reported [7,25–27], in contrast to a more recent study of 28 patients from Australia, in which a lower mortality rate of 7.1% was observed [22]. Albeit these differences, human and animal infections with *Chromobacterium* have a major impact in public health, justifying the growing number of studies in the last few years considering the pathogenesis and treatment regimens [28–30].

The first case of *C. violaceum* infection was reported in water buffalos in the Philippines (Woolley, 1905) [31], whilst its potential pathogenicity to humans was first described in 1927, in Malaya (currently known as Malaysia) [32]. This was followed by other case reports in different countries, comprising around 150 cases with available location data worldwide, of which, 50 cases (33.3%) occurred in western Pacific (Cambodia, China, Australia, Singapore, Vietnam, Laos, Japan, Korea, Malaysia, Papua New Guinea and Solomon Islands), 2 cases (1.3%) in the Persian Gulf, 1 case (0.7%) in Europe, 46 cases (30.7%) in Americas, 29 cases (19.3%) in southeast Asia (Nepal, Thailand, India and Sri Lanka) and Africa with 22 cases (14.7%) [2,5,7,22,23,33–36]. However, these numbers must be underestimated due to the rapid evolution of the disease.

The major source of infection is through a cutaneous trauma or injury, as well as through ingestion of contaminated seafood or water. However, other routes of infection include scuba diving or near drowning, road traffic and airplane accident, and, importantly, cases of nosocomial infections have also been reported [27,34,36–40]. In this respect, Anah and colleagues [41] reported the isolation of *C. violaceum* from 10 inborn neonates. Recently, this microorganism was found in water sampled in hospitals [42,43]. The description of hospital-acquired infections warrant attention due to the life-threatening potential associated with this pathogen and the fact that most clinicians are not familiar with it.

Chromobacterium violaceum infections were recently reported in a 14-year-old boy who was diagnosed with necrotizing faciitis, in the United States [44], and in Italy, in a 14-year-old boy diagnosed with cervical lymphadenitis [13]. Recently, the number of cases in Nepal has increased [25–27,35,36]. This may be associated with progresses in laboratory diagnosis or climate change, which has led to the spread of the microorganism to other geographical locations [7]. Pant and colleagues [36] reported a case of urinary tract infection caused by *Chromobacterium violaceum* in a patient with kidney disease. In this same direction, Pant and Sharma [27] and Ma and colleagues [45], from China, have also published similar cases, whilst Swain and colleagues [46], from India, and Pant and colleagues [25] have shown urinary tract *Chromobacterium* infection in immunocompetent patients. Furthermore, a 43-year-old diabetic woman with a history of urinary catheterization and a 12-year-old girl were also diagnosed with bacteriuria associated with *C. violaceum* infection in India [34,47]. Ansari and colleagues [26] reported the first case in Nepal involving isolation of *C. violaceum* from wound sepsis that led to death due to progression to septic shock and multiorgan failure. Parajuli and colleagues [35] also recently reported a case of wound-related sepsis in a 36-year-old female; however, in this case complete recovery after antimicrobial therapy was achieved. Cases of septicemia have also been reported in India in the last few years. In only one of these cases successful treatment was achieved [48–50]. These data indicate the importance of early diagnosis of the infection and administration of proper antimicrobial treatment on the basis of susceptibility tests, even after empirical use of antimicrobial agents, to avoid the infection to progress to fatal sepsis [29].

In addition, infection has been shown to be severe in immunocompromised and malnourished patients [51]. Recently, the first case of *Chromobacterium violaceum* infection was reported in a

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