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Chromobacterium violaceum genome: molecular mechanisms associated with pathogenicity

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ABSTRACT. *Chromobacterium violaceum* is a versatile, Gram-negative β-protebacterium that grows in a variety of ecosystems in tropical and subtropical areas, such as the water and borders of the Negro River, in the Amazon region of Brazil. Although it is a saprophyte and is generally considered non-pathogenic, sporadic cases of human infection have been described, mainly in young children and in immunodeficient individuals. Although rare, infections with *C. violaceum* are characterized by rapid dissemination and high mortality. With the complete genome sequence of *C. violaceum* now available, a detailed description of the molecular arsenal required for this bacterium's remarkable versatility has been revealed. Most importantly, a more detailed picture of its biotechnological properties, including the characteristic violacein pigment, has emerged. The complete genome sequence also enabled us to make a thorough examination of the repertoire of genes encoding probable virulence factors, which determine the potential for pathogenesis. We

described a number of genes involved in infectious processes, such as host cell adhesion, "contact-dependent secretion" of factors that promote cell invasion, as well as other virulence factors, such as cytolytic proteins. We also described genes involved with the synthesis of lipopolysaccharides and proteoglycan, known to elicit the synthesis of pro-inflammatory cytokines and involved in the detoxification process, which may contribute to the evasion of the bacteria from the host immune response.

Key words: *Chromobacterium violaceum*, Pathogenicity, Virulence, Genome

INTRODUCTION

Chromobacterium violaceum (*Cv*) appears to be an opportunistic pathogenic bacterium, which affects humans and animals in subtropical and tropical areas. The low infectious capability of *Cv* is evidenced by the fact that rivers such as Negro River, in the Amazon region of Brazil, where the bacteria is highly abundant, are sources of drinking water, without the occurrence of widespread infection among the local population. Since the first human case, described in Malaya in 1930, other reports of human infection have been described sporadically, mainly in young children and in immunodeficient individuals. Among the immunodeficient individuals, cases in HIV-infected individuals and in children with chronic granulomatous disease, have been described. Infections caused by *Cv* are associated with skin lesions, bacteremia and septic shock syndrome (Duran and Menck, 2001). In agreement with its pathogenic potential, several genes involved with host cell adhesion and invasion as well as transporter systems for virulence factors have been found in the *Cv* genome (Brazilian National Genome Project Consortium, 2003). Four distinct classes of secretion pathways that deliver virulence factors to their sites of action have been identified in Gram-negative pathogens. Components of type I, II and III secretion systems have been identified in the *Cv* genome. Two of these three secretion pathways show an evolutionary relationship with the biogenesis apparatus responsible for the assembly of bacterial surface organelles. The machinery of type II and III secretion pathways shares sequence similarities with proteins involved in the assembly of pili and flagella, respectively (Lory, 1998). Type I secretion systems are composed of ABC transporters, which are involved with secretion of various toxins, mainly RTX-containing proteins, such as hemolysins. The genome of *Cv* appears to encode various types of cytolytic proteins. In addition to genes related to cell adhesion, invasion and transport of virulence factors, we also found *Cv* genes involved with biosynthesis of lipopolysaccharides (LPS) and proteoglycans. It is well established that the excessive production of pro-inflammatory cytokines and excessive activation of the immune system are important mechanisms involved in host pathogenesis during systemic infection with Gram-negative bacteria and in the septic shock syndrome. LPS and proteoglycan act synergistically in inducing pro-inflammatory cytokines, as well as other acute phase inflammatory proteins. We also identified genes active in detoxification, particularly those that are used in the reduction of free radicals; these could be involved in bacterial evasion from the host immune system, and were thus potentially considered bacterial virulence factors. Finally, we

identified genes involved in the synthesis of violacein, which has also been reported as a possible factor involved in pathogenesis caused by *Cv*.

GENES ASSOCIATED WITH HOST CELL ADHESION (TYPE IV FIMBRIAE/ TYPE II SECRETION SYSTEM)

Gram-negative bacteria assemble a number of adhesive structures that are involved in recognition and adherence to the host cell. Pili, or fimbriae, are hair-like structures that radiate out from the bacterial surface and present adhesive moieties, known as adhesins, which recognize specific host cell receptors (Thanassi and Hultgren, 2000). Studies with the pathogenic bacteria *Neisseria meningitidis* and *Pseudomonas aeruginosa* (*Pa*) indicate pili as an important cell-associated virulence factor that plays a crucial role in mediating bacterial adherence and colonization (Darzins and Russell, 1997; Tonjum and Koomey, 1997).

Adhesive pili are assembled by at least three distinct pathways. First, biogenesis of a superfamily of more than 30 pili and non-pili adhesion molecules requires outer membrane proteins, known as usher, that work together with periplasmic chaperones as part of the chaperone/ usher pathway (ex; type I pili coded by the *fim* gene cluster). A second pathway for assembling adhesive pili is exemplified by the type IV system. These are long, polarly localized pili responsible for twitching motility and bacterial aggregation, as well as adhesion (Thanassi and Hultgren, 2000). The chaperone-usher pilus family and the type IV pilus family are unrelated to each other, but the machinery for pilus assembly is highly conserved (Donnenberg, 2000). Twitching motility describes flagella-independent surface motility, which has been described in a wide range of bacteria, including *Pa* and *Neisseria gonorrhoeae*. It occurs on wet surfaces and is important for host colonization and other forms of complex colonial behavior, including the formation of biofilms and fruiting bodies (Mattick, 2002). Twitching motility is mediated by type IV pili and it appears to be largely restricted to Gram-negative bacteria, mainly the β, γ, δ subdivisions of the Proteobacteria (Mattick, 2002). Genes involved in the assembly of type IV pili machinery help mediate bacterial pathogenesis, since twitching motility is important for infection by organisms such as *Pa* (Comolli et al., 1999).

The components of the type IV pili are similar to the 13 proteins of the type II secretion pathway. There is a gene-per-gene correspondence among these components (see, Lory, 1998). Adhesion of bacteria to the host can initiate signaling events leading to invasion by the bacteria, blockage of uptake into phagocytic cells or the formation of a specialized niche on the surface of the host. Recent work has suggested that pili are not only involved in invasion events, required during initial contact, but that they are also involved in various other functions during infection, depending on the bacterial mechanism (Thanassi and Hultgren, 2000).

Chromobacterium violaceum has several genes involved in the assembly of type IV pili machinery. The relatively well-studied pathogenic bacteria *Pa* can be used as model to study this function in *Cv* since these bacteria have a close phylogenetic relationship and we found a high degree of homology among their genes. Studies on type IV pilus biogenesis from *Pa* indicated that as many as 40 genes may be required for its production (Darzins and Russell, 1997). The *Pa pil* genes were named *pilA* to *Z* (Mattick et al., 1996; Darzins and Russell, 1997), but not all of them were found in the *Cv* genome. The genes found in the *Cv* genome are: *pil B*, *C*, *D*, *E1*, *E2*, *E3*, *M*, *N*, *O*, *P*, *Q*, *R*, *S*, *T*, *U1*, *U2*, *V*, *W*, *Y1*, *X*, *Z*, and some others without known function. The function of most of them was inferred based on their homology with the *Pa* genes.

Most of these genes are clustered in the *Cv* genome, and in some cases the clusters are similar to those observed in the *Pa* genome. Some genes are repeated in different regions of the *Cv* genome, suggesting that they could be a result of horizontal gene transfer. For instance, the gene *pilE* repeats three times, one repeat is inserted in a region with similarity to *Neisseria meningitidis* genes and the other in a region with high similarity to *Ralstonia solenacearum.* According to Mattick (2002), the exchange of subunit genes appears to have occurred naturally; this appears to be the case in *Pa* and *D. nodosus*, which have unusual pilins that almost certainly have been derived by lateral transfer from other species.

Lory (1998) summarized the relationships between the type II secretion machinery, composed of 13 genes and type IV pilus biogenesis, composed of 11 genes. Based on homology between the genes belonging to the type II secretion and type IV pilus biogenesis, which is present in *Cv*, we can suggest that at least part of the type II secretion machinery is encoded in the *Cv* genome. In a comparison with the *Pa* genome, we were unable to find some important type IV pilus biogenesis genes, mainly genes involved with a signal transduction network (*pilGHIJKl*). However, these latter genes have significant similarities with chemotaxis proteins, and some of them are present in the *Cv* genome; we can thus speculate that the product of the genes classified as chemotaxis proteins could play the same role.

Mattick (2002) reviewed all species available and mainly defined the genes required for assembly of type IV pili. *Cv* has all of them in its genome; some are in the same organization observed in *Pa*: cluster *pilBCD*, *pilMNOPQ*, *pilT*, *U2*. PilB and PilT are nucleotide-binding proteins, PilC is an inner membrane protein, PilD is a prepilin peptidase and methylase, and PilQ proteins form gated pores in the outer membrane, through which the pilus is thought to extrude. The genes present in PilQ operon are also required for twitching motility.

PilR and PilS, clustered in *Cv*, as they are in *Pa*, are a two-component sensor-regulator that controls the transcription of PilA, the gene that codes the monomers of the pilin structural subunits (Hobbs et al., 1993). Interestingly, gene *pilA* of *Pa* was not found in the *Cv* genome, instead we have identified *FimA*, which is similar to *F17*, a protein fimbria family found in some *E. coli* strains that cause intestinal and extraintestinal disease in animals and humans. Some members of the F17 family are associated with septicemia in cattle (Cid et al., 1999). The complete cluster found in other organisms includes *fimA-I* (Thanassi and Hultgren, 2000), but in the *Cv* genome we found only the genes *fimA*, B, and D, and a hypothetical conserved export protein. These genes include a major structural component of fimbrial adhesin similar to *E. coli* gene F17 (*fimA*), the chaperone (*fimB*) and the usher proteins involved in the exportation and assembly of the subunits through the outer membrane (*fimD*). The cluster pilVWXY1 present in both *Cv* and *Pa*, probably forms the base structure for the pilus fiber, as suggested by Mattick (2002). PilZ is transcriptionally coupled to holB, which encodes the δ subunit of DNA polymerase III, perhaps linking the assembly pili to the cell cycle (Alm et al., 1996).

GENES RELATED TO CELL INVASION (TYPE III SECRETION SYSTEM)

Chromobacterium violaceum has three large clusters related to the type III secretion system (TTSS), two containing genes homologous to *Salmonella* spp., belonging to *Salmonella* pathogenicity island-1 (SPI1), and one with genes similar to *Yersinia* spp. Electron micrographs reveal that the type III secretion apparatus resembles a syringe. This syringe is thought to secrete effector proteins from the bacterium, and these proteins stimulate dramatic cytoskeletal

rearrangements in eukaryotic host cells (Finlay et al., 1991; Kubori et al., 1998). These membrane ruffles facilitate the engulfment of the bacteria by eukaryotic cells. The first cluster is composed by *invFGEABCIJ-spaOPQRS* genes in the *Cv* genome and is located in *Salmonella* within the SPI1, with exactly the same gene organization, missing only the invH gene in *Cv* (Figure 1). In these clusters, all but one gene has counterpart homologous genes in other pathogenic species, such as *E. coli* O157:H7 and *Yersinia enterocolitica*, the exception is the *invH* gene. This gene encodes for an outer membrane lipoprotein required for proper localization of InvG (Daefler and Russel, 1998). We found 29 genes in the *Cv* genome encoding putative lipoproteins, most were predicted to be outer membrane proteins by pSORT, and some of them have no consistent homologies in Genbank. Therefore, any of these lipoproteins with unknown function could correspond to the *inv*H gene in *Cv*.

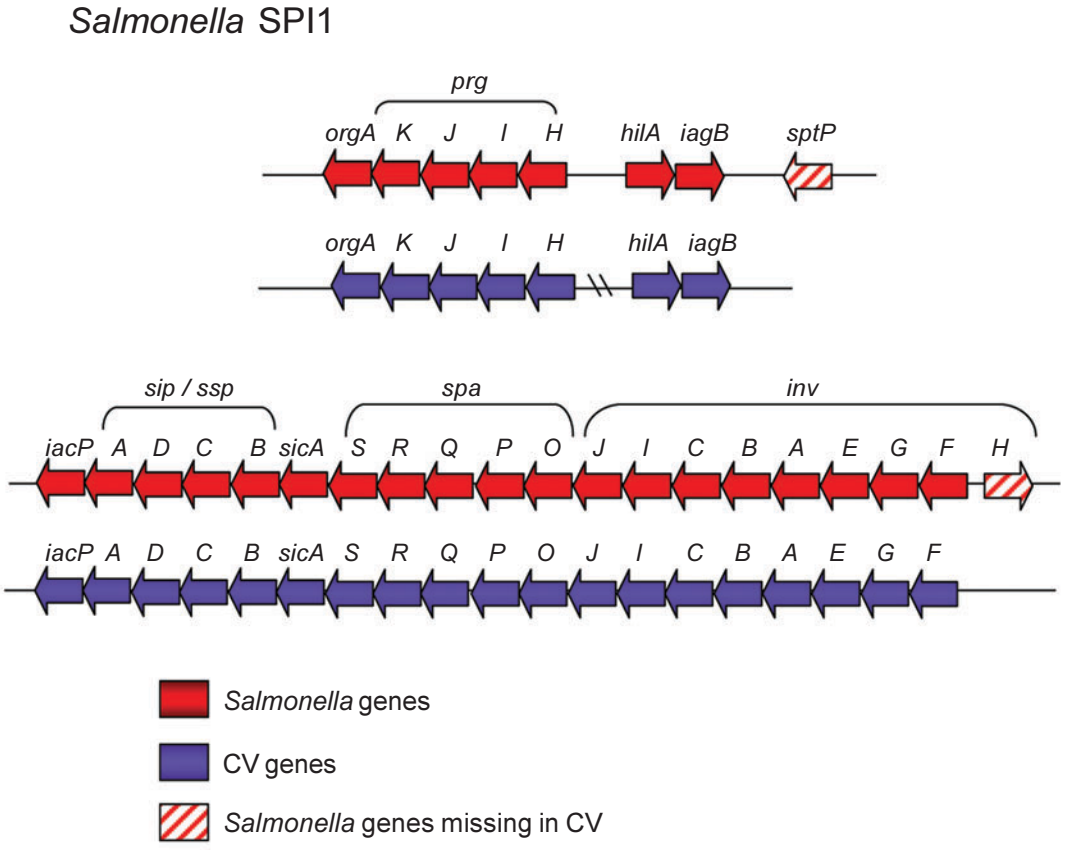

Figure 1. Schematic organization of the Inv-Spa cluster showing genes found in *Chromobacterium violaceum* (CV) and genes of *Salmonella* pathogenicity island-1 (SPI1). Arrows indicate the transcriptional orientation.

Another cluster also located in the SPI1 is composed of the *prgHIJK-orgA* genes, which are identical in *Cv* and *Salmonella typhimurium* (Klein et al., 2000). The core of the complex formed by correspondent proteins, probably found embedded in the inner membrane and crossing the periplasmic space, is comprised of PrgH and PrgK, while the barrel of the syringe of the secretion apparatus is predominantly composed of PrgI, a protein that requires

PrgJ for its stability (Kimbrough and Miller, 2000). All type III secretion systems include a secretein family of outer membrane proteins, possibly forming a channel through which the needle of the type III apparatus passes. In the case of TTSS-1, the protein InvG probably serves this purpose (Daefler and Russel, 1998).

The proteins that utilize the TTSS pathway have evolved a complex system, whereby synthesis of some individual secreted proteins is immediately followed by association with specific chaperones (Wattiau et al., 1996). Regarding secreted proteins and their chaperones involved in the invasion of host cells, we found the cluster *sicA-sipBCDA-iacP* in *Cv*, which is closely related to *Salmonella* and it is located immediately upstream of the *spa*S gene (Figure 1). Chaperones have been identified in other TTSS and they are required for the secretion, stabilization, and/or translocation of effector proteins (Wattiau et al., 1994). In SPI1, the chaperone protein SicA guides the secreted targets, SipB and SipC to the translocation machinery (Wattiau et al., 1996). SipB encodes a secreted protein required for the invasion of *S. typhimurium* and *S. typhi* into tissue culture cells (Kaniga et al., 1995a). Like SipB, SipC is required for the invasion of *Salmonella* and for translocation of SipB and SptP into eukaryotic cells (Fu and Galán, 1998). Unlike SipB, and SipC, SipD does not appear to enter eukaryotic cells, but it is required for the translocation of SipB, SipC and other effector proteins (Hardt and Galán, 1997). As for SipA, it is the largest *Salmonella* protein in this cluster and it is thought not to be essential for invasion. SipC is essential for the process, while SipA enhances its efficiency. The accessory protein IacP, found in this cluster, is involved in the post-translational modification of exported proteins (Kaniga et al., 1995b).

In *S. typhimurium*, associated with the *prg* cluster, there is a gene coding for an effector protein termed SptP (secreted protein tyrosine phosphatase); the corresponding gene was not found in *Cv*, nor was its cognate chaperone SicP (*Salmonella* invasion chaperone) (Kaniga et al., 1996). The SptP has a modular architecture, composed of two domains, the N-terminal, homologous to exotoxin S of *Pa* and YopE protein of *Yersinia* spp., involved in depolymerization of the actin microfilament network of host cells (Rosqvist et al., 1991). The C-terminal domain is homologous to the catalytic domain of tyrosine phosphatases, including the *Yersinia* YopH protein, which prevents phagocytosis of *Yersinia* by macrophages (Andersson et al., 1996). In *Cv* there is at least one gene with a C-terminal domain homologous to YopH but the N-terminal has no significant homologies in Genbank (CV0974).

Secretion of proteins via the TTSS pathway is not a constitutive process; instead, export is triggered by exogenous signals (Lory, 1998). The TTSS is activated when a pathogen comes into contact with an eukaryotic cell and, hence, has been called "contact-dependent secretion". Once delivered into the host cell, effector proteins modulate host cell functions to the bacterium's advantage (Cornellis and Van Gijsegem, 2000). The TTSS is responsible for cytoplasmic delivery of proteins into the host cells by a variety of human and plant pathogens (Menard et al., 1996; Galán, 1996; Yahr et al., 1996; Cornellis and Wolf-Watz, 1997; Lee, 1997; Baker et al., 1997). Invasion gene expression also requires the central regulator HilA, which was also found in *Cv*. Invasion into epithelial cells and induction of apoptosis of macrophages is absolutely HilA dependent (Bajaj et al., 1995). Expression of *hilA* is activated by SirA (*Salmonella* invasion regulator), but it is not known how environmental signals stimulate its expression or activity. A mutation in *sirA* dramatically reduces the invasiveness of *S. typhimurium* (Johnston et al., 1996). We were not able to identify *sir*A in *Cv* and, since it is a key molecule to activate HilA, lack of *sir*A might dictate a noninvasive phenotype usually

observed in *Cv*. However, *sir*A belongs to a family of two-component regulators and *Cv* has around 47 genes in this family, some of them with no significant homologies in Genbank. Due to the high variability of regulators, one of these putative regulators found in *Cv* may have a role similar to SirA. It is well established that transcriptional regulation of TTSS-1 genes is a primary mechanism for controlling the production of TTSS-1 factors in response to environmental signals (Lucas et al., 2000). Therefore, we speculate that the difference between an invasive pathogenic *Salmonella* and an opportunistic *Cv*, which carry several homologous genes for host cell invasion, might result from different regulatory mechanisms of gene expression in these bacteria.

Chromobacterium violaceum has another large cluster involved in the expression of TTSS machinery components, and almost all genes in this cluster had the highest similarity with *Yersinia* spp. genes. This machinery consists of around 20 polypeptides, located predominantly in the cytoplasmic membrane, constituting an inner membrane channel, which is energized by an ATP-binding protein, YscN, in *Yersinia*, also present in *Cv*. At least one outer membrane component is necessary in this system; this protein, homologous to GspD, is also a member of the type II pathway (Lory, 1998). Proteins secreted by this pathway are translocated without an apparent periplasmic intermediate. According to Plano et al. (2001), there are 11 genes involved in the expression of TTSS structural components, which are broadly conserved in several pathogens, such as *Yersinia pestis*, *Salmonella* SPI1 and *Pseudomonas* spp. Members of the *yscC*, *D*, *J*, *L*, *N*, *Q*, *R*, *S*, *T*, *U*, and *V* gene families have counterparts in almost every TTSS (Hueck, 1998). These genes are similar to genes involved in flagellar assembly. Concerning the TTSS, *C. violaceum* has all the essential genes to build the apparatus of secretion. Moreover, like *Yersinia*, *Cv* has two other clusters involved specifically in the assembly of the flagellum. Genes encoding the secreted and structural components that make up each individual TTSS are generally found on a single plasmid, or within a distinct pathogenicity island in several pathogenic bacteria, suggesting that these genes are inherited as a single unit.

The structure of TTSS from *Salmonella* resembles the flagellar apparatus, and consists of a needle protruding from the bacterial surface connected to a base that is surrounded by two upper (outer membrane) and two lower (inner membrane) rings (Donnenberg, 2000). The structure of a system from *Shigella* also reveals a protruding needle, which is attached to a 10 nm neck, sufficient to pass through both membranes, and to a large cytoplasmic bulb (Donnenberg, 2000) (Figure 2). An appendage forms a bridge between the bacteria and the host cell, and it is composed of a protein (EspA) that is secreted by the type III apparatus (Donnenberg, 2000). In *Cv*, we probably have a mix of these structures, needle and appendage. The majority of the machinery component genes in *Cv* are more related to *Yersinia*, which forms a needle-like structure. However, *Cv* has also two copies of the gene encoding EspA, with the highest similarity to EPEC. In *Salmonella*, this gene belongs to *Salmonella* pathogenicity island-2 and is specifically expressed upon entry into mammalian cells, and is required for intracellular growth in host cells *in vivo* and *in vitro* (Cirillo et al., 1998).

Sequence similarities suggest that although many of the structural components of the TTSS apparatus are conserved among the different bacterial species that use TTSS; the proteins delivered by these pathogens are quite diverse (Galán and Collmer, 1999). There are at least two functional categories of secreted targets; they are proteins involved in secretion and proteins with putative effector functions in the host cell (Collazo and Galán, 1996). The proteins InvJ, SpaO, SipB, and D are in the first group; the encoding genes for all of them are also found

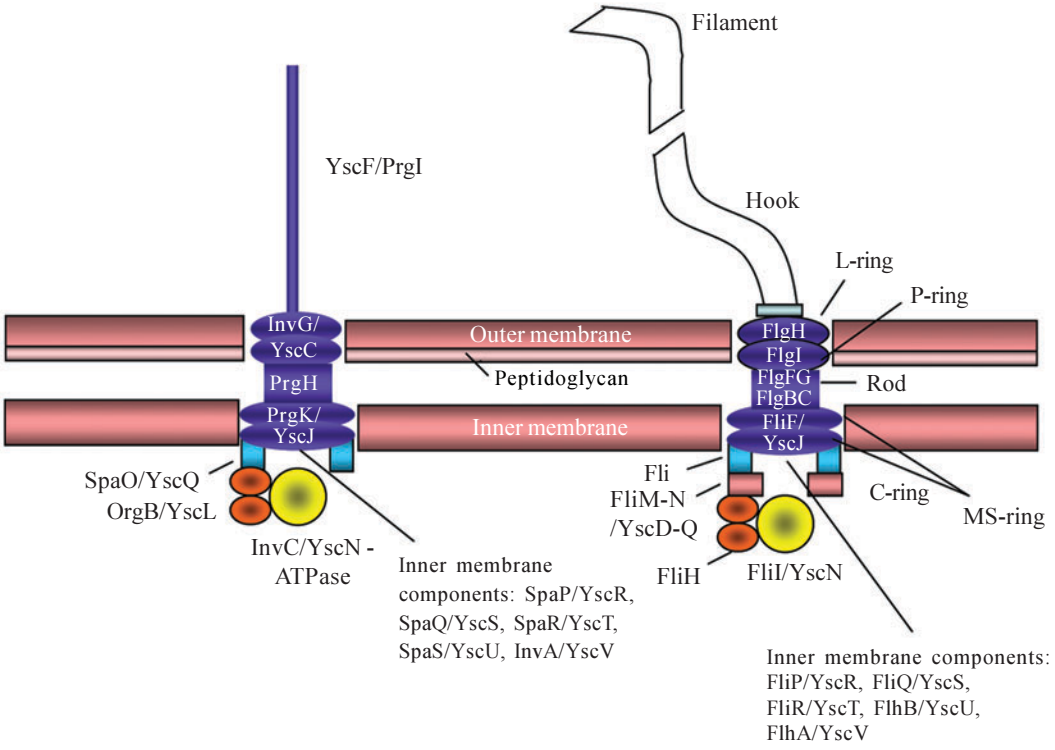

Figure 2. Schematic structure of type III secretion system apparatus indicating proteins that composed each component. The names are according to *Salmonella* spp./*Yersinia* spp.

in the *Cv* genome. There is a great diversity in the effector group. Exotoxins secreted by the type III system do not possess a secretion signal; however, small stretches in the amino-terminal domains are involved in the secretion (Wattiau et al., 1996). In *Yersinia*, TTSS allows translocation of a range of effector proteins (YOPs) that downregulate the responses of host phagocytic cells to infection. We were unable to identify many effectors for this system in *Cv*, as these are generally divergent. However, we found some genes in *Cv* with similarities to secreted proteins of the TTSS: *yopE*, *yopB*, *popB*, and *sopE*. We were not able to find any gene encoding effector proteins of plant pathogens related to the *avr* families. Among the effector proteins, YopE disrupts the actin cytoskeleton, facilitating cell invasion by *Yersinia* (Donnenberg, 2000). SopE is a guanine nucleotide exchange factor that is injected into the host cell cytoplasm, where it can directly stimulate actin cytoskeletal rearrangements. It acts as a guanidine exchange factor for members of the Rho subfamily of small GTPases. The *sopE* gene mutants exhibit less extensive actin cytoskeletal rearrangements upon entry into epithelial cells than do wild-type *Salmonella*. YscD is a protein required for proper EspB and EspD secretion. YscD from *Escherichia coli* O:157 shows sequence homology with chaperone proteins from other type III secretion pathways, such as YopB/D (*Yersinia*) and PcrH (*Pseudomonas*). These proteins are involved in the stabilization of secreted protein, preventing unproductive aggregation, and they also have a role in the presentation of the targeting signal to the secretory machinery. A previous study has shown that YopB of *Yersinia* spp. is essential for the translocation of Yop effectors across the eukaryotic plasma membrane.

GENES RELATED TO LIPOPOLYSACCHARIDE (ENDOTOXIN) AND PEPTIDOGLYCAN BIOSYNTHESIS

LPS is one of the main components of Gram-negative bacteria cell walls and it is largely responsible for the activation of host immune cells and the induction of pro-inflammatory cytokines during infection with these bacteria (Karibian et al., 1994; Shade et al., 1999). LPS is a complex glycolipid, which appears to be an important factor involved in host pathogenesis. The carbohydrate moiety of LPS, especially the terminal O-antigen is a main target for antibody response, and thus may be involved with protective immunity to infection in individuals that have been previously exposed to certain species or subtypes of bacteria. Secondly, lipid A, also named endotoxin, is known for stimulating host cells to release high levels of pro-inflammatory cytokines, which are an important component of the septic shock observed in individuals with systemic bacterial infection (Ingalls et al., 1998). It is noteworthy that most of the cases of disease caused by *Cv* are associated with sepsis (Midani and Rathore, 1998). We found 32 genes that are potentially involved with the synthesis of LPS. Only one is not involved in the synthesis of O-antigen, core region and lipid A. Among the 12 genes involved with the synthesis of the LPS core region, six are clustered in the *Cv* genome (Figure 3). All six genes involved in the synthesis of lipid A have the highest similarity with genes from *N. meningitidis* subtypes (Figure 3), consistent with the structural similarity of lipid A from *Cv* and *N. meningitidis* (Hase and Reitschel, 1977; Kulshin et al., 1992).

Peptidoglycan is present in the cell wall of both Gram-positive and Gram-negative bacteria. Similarly to LPS, peptidoglycan is known to elicit the synthesis of pro-inflammatory cytokines (Weidemann et al., 1997; Rietschel et al., 1998) and synergize with LPS (Rietschel et al., 1998; Yang et al., 2001); thus it is considered to be involved in septic shock in patients undergoing septicemia. We found 25 genes involved in the synthesis of peptidoglycan in the *Cv* genome, 11 of which had the highest homology with genes from *N. meningitidis*. Among the 25 genes involved in the synthesis of peptidoglycan, nine are organized in a cluster; six of these had the highest homology to genes from *N. meningitidis* (Figure 3).

HEMOLYSINS, COLICINS AND OTHER TOXINS PRODUCED BY *CHROMOBACTERIUM VIOLACEUM*

Hemolytic activity has been detected in clinical isolates, as well as in a soil isolate, of *Cv* (Miller et al., 1988). Thirteen ORFs showing homology with previously identified hemolysins or related to hemolysin production or secretion are present in the *Cv* genome. Several genes encoding protein members of the RTX family, a group of exoproteins of Gram-negative bacteria that includes cytolytic toxins (hemolysins or leukotoxins), metallo-proteases and lipases, were found in *Cv*. A specific RTX secretory apparatus, which belongs to the type I secretion system, consists of an inner membrane protein (RTX protein B), which provides energy for protein export through ATP hydrolysis, and a transmembrane channel consisting of an RTX D protein and the outer membrane TolC-like protein. RTX B exporter proteins are a distinct branch of the ABC superfamily of membrane transporters and RTX D are members of the MFP (membrane fusion protein) family of proteins. The *Cv* genome contains several genes homologous to the components of the *E. coli* A-hemolysin (HlyA) secretion system (Gentschev et al., 2002). Among them is the RTX A gene product (CV0311), which contains the tandem repeats consist-

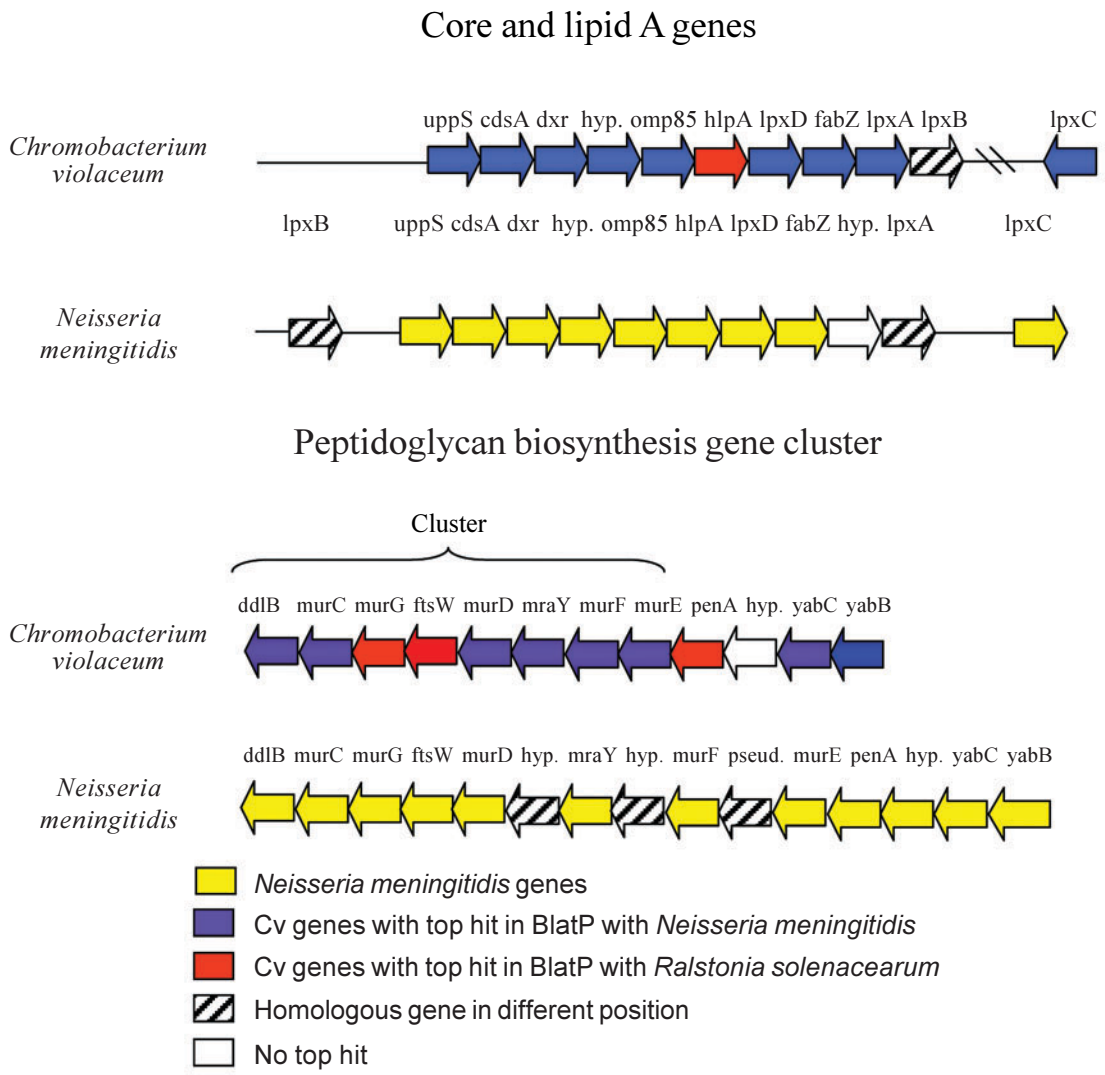

Figure 3. Gene organization in clusters involved in lipid A, peptidoglycan and O-antigen biosynthesis.

ing of the RTX nonamer signature, which is present at the amino-terminal end of the export targeting protein. The RTX C gene (CV01917) was found linked to a hemolysin A gene (CV01918), and it encodes a product that participates in the activation of cytolisin. These genes present high homology to the corresponding proteins from *Serratia marcescens*, *Yersinia pestis* and *Proteus mirabilis*, but show low homology to the *E. coli* RTX A and C genes. Genes homologous to RTX B (CV0513) and RTX D (CV0516), which are also present in the genome of *Xylella fastidiosa*, *Neisseria meningitis*, *Actinobacillus* sp., and *Ralstonia solacenarum* were found to be associated in the *Cv* genome, as in these other organisms. Besides these, thermolabile hemolysins, with homology to phospholipase/lecithinase, also present in *V. cholerae* (CV0360 and CV0362) and to sphingomyelinase from *Staphylococcus aureus* (*CV*3275), were found. Another potentially membrane-damaging toxin homologous to hemolysin III (CV3342) from *Listeria monocytogenes* was also found.

ENZYMES INVOLVED IN DETOXIFICATION

Nitrogen and oxygen free radicals are released by interferon-gamma-activated macrophage effector cells from the vertebrate host and are important components of host resistance against microorganisms (Nathan, 1982, 1995; Hickman-Dacis et al., 2001). Individuals with the chronic granulomatous disease of childhood syndrome are severely compromised in their respiratory burst, with little generation of hydrogen peroxide and other oxygen products, and they have recurrent infections with catalase positive bacteria (Gallin et al., 1992). It is noteworthy that different studies show that individuals with the chronic granulomatous disease have an enhanced susceptibility to *Cv* (Macher et al., 1982; Sorensen, 1985), which is catalase positive. Furthermore, *Cv* has been found to be an opportunistic pathogen in individuals infected with HIV (Fombuena et al., 1998), which have diminished interferon-gamma production and consequently compromised release of free radicals by host immune cells. We found various genes that are potentially involved in the process of detoxification (xenobiotic metabolism), among them, catalase (CV3549), various peroxidase genes, two superoxide dismutase genes (CV0867 and CV2504) and others, that contribute to reduce harmful free radicals. A comparison study of virulent and avirulent strains of *Cv* shows that the virulent strain was more resistant to phagocytosis and intracellular killing by human polymorphonucleocytes (Miller et al., 1988). The clinical isolate showed a 30% higher superoxide dismutase activity and a five-fold higher catalase activity than the activities observed in soil-isolated strains. Since these activities can be used as mechanisms of bacteria evasion of the host immune system, the genes involved in detoxification were also considered here as pathogenicity and/or virulence factors of *Cv*.

GENES RELATED TO THE SYNTHESIS OF VIOLACEIN

Violacein is the main pigment produced by *Cv*. It is a purple-colored, broad-spectrum antibacterial pigment that has a dimeric structure composed of 5-hydroxyindole, oxindole and 2-pyrrolidone subunits formed by the condensation of two modified tryptophan molecules, the production of which has been shown to be regulated by quorum sensing (McClean et al., 1997). The violacein biosynthetic gene cluster from *Cv* was previously characterized by DNA sequencing, mutagenesis, and chemical analysis of the synthetic pathway (August et al., 2000). Violacein has been suggested to display several types of properties, including antibiotic, trypanocide, tumoricide, and antiulcerogenic (Duran et al., 1996, 2003). However, most of its activity may be linked to its generalized cytotoxic properties (Andrighetti-Frohner et al., 2003). It has been shown that a decrease in the cytotoxic activity by transformation of groups in the molecule also proportionally decreases its antitumoral activity (Bromberg and Duran, 2001). Thus, a possible role of violacein in human pathogenesis could be related to its cytotoxic activity, which has been shown to induce apoptosis, but not necrosis, in fibroblasts (Melo et al., 2000). However, the likely association between violacein and the pathogenicity of *Cv* has been challenged by reports of isolation of non-pigmented *Cv* strains in at least one case of human infection (Sorensen et al., 1985; Midani and Rathore, 1998).

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