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Tolerance to stress and environmental adaptability of *Chromobacterium violaceum*

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ABSTRACT. *Chromobacterium violaceum* is a Gram-negative bacterium, abundant in a variety of ecosystems in tropical and subtropical regions, including the water and borders of the Negro River, a major component of the Amazon Basin. As a free-living microorganism, *C. violaceum* is exposed to a series of variable conditions, such as different sources and abundance of nutrients, changes in temperature and pH, toxic compounds and UV rays. These variations, and the wide range of environments, require great adaptability and strong protective systems. The complete genome sequencing of this bacterium has revealed an enormous number and variety of ORFs associated with alternative pathways for energy generation, transport-related proteins, signal transduction, cell motility, secretion, and secondary metabolism. Additionally, the limited availability of iron in most environments can be overcome by iron-chelating compounds, iron-storage proteins, and by several proteins related to iron metabolism in the *C. violaceum* genome. Osmotically inducible proteins, transmembrane water-channel, and other membrane porins may be regulating the movement of water and maintaining the cell turgor, activities which play an important role in the adaptation to

variations in osmotic pressure. Several proteins related to tolerance against antimicrobial compounds, heavy metals, temperature, acid and UV light stresses, others that promote survival under starvation conditions, and enzymes capable of detoxifying reactive oxygen species were also detected in *C. violaceum*. All these features together help explain its remarkable competitiveness and ability to survive under different types of environmental stresse.

Key words: Adaptability, Secondary metabolism, Stress tolerance, *Chromobacterium*

INTRODUCTION

Chromobacterium violaceum was first described at the end of the 19th century (Boisbaudran, 1882), after which several reports confirmed its presence in the soil and water of tropical and subtropical regions. Its most notable characteristic is the production of violacein, a purple pigment first isolated in 1944 (Strong, 1944), and chemically characterized a few years later (Ballantine et al., 1958). In Brazil, the abundance of this bacterium in the water and on the borders of the Negro River, in the Amazon Region, and the therapeutic properties of the violacein have been reported since the end of the 1970s (Caldas et al., 1978; Durán et al., 1983, 1989, 1994; Caldas, 1990; Durán, 1990; Souza et al., 1999; Melo et al., 2000; Leon et al., 2001). The competitive success of *C. violaceum* in several microbial communities, as well as the wide range of its habitats, is certainly indicative of a remarkable ability to survive under different environmental stresses. Indeed, evidence of a wide range of genes related to adaptability was revealed with the complete genome sequencing of free-living *C. violaceum* (Vasconcelos et al., 2003).

GENERAL MECHANISMS RELATED TO STRESS TOLERANCE AND ADAPTABILITY

As with other free-living microorganisms, *C. violaceum* is exposed to a series of variable conditions, such as different sources and abundance of nutrients, changes in temperature, toxic compounds, and UV rays. These variations require fast adaptive responses, usually triggered by transcriptional activation of specific genes. A set of genes controlling basal transcriptional mechanisms, such as RNA polymerase and common sigma factors, e.g., σ^{70} (*rpoD*, CV3762), σ^{54} (*rpoN*, CV3332), σ^{32} (*rpoH*, CV4206) and σ^{38} (*rpoS*, CV3682) are present in *C. violaceum*. Furthermore, a large number of transcriptional activators and repressors that interact with alternative sigma factors involved in bacterial cell response to stress, such as those belonging to LysR (CV0061, CV0270, CV0509, etc.), AraC (CV1314, CV1825, CV2101, etc.), TetR (CV0385, CV0711, CV1077, etc.), among others are also present. For additional information about transcriptional factors and regulation in *C. violaceum*, see Silva et al. (2004).

The *C. violaceum* genome has a high proportion (6.4%) of ORFs associated with signal transduction mechanisms (COG category T), when compared with other free-living sequenced microorganisms (Vasconcelos et al., 2003), which reflect an enhanced adaptability to

diverse environmental conditions. Additionally, 5.8% (257 of 4,431 valid open reading frames, ORFs) of the genes of *C. violaceum* encode transcriptional regulators, similar to the proportions found in other versatile microorganisms capable of colonizing soil, water, plant, and animal tissues, such as *Pseudomonas aeruginosa*, *E. coli* and *Bacillus subtilis*, which contain 8.4, 5.8 and 5.3% of the ORFs involving regulatory proteins, respectively. In contrast, such genes constitute only 3.0% and 1.1% of the genome in the specialized pathogens *Mycobacterium tuberculosis* and *Helicobacter pylori*, respectively (Blattner et al., 1997; Kunst et al., 1997; Hancock et al., 1998; Cole et al., 1998; Stover et al., 2000).

The ecological versatility of *C. violaceum* is also confirmed by the identification of 204 ORFs related to energy metabolism, including fermentation, aerobic and anaerobic respiration (Vasconcelos et al., 2003; Creczynski-Pasa and Antônio, 2004), allowing this bacterium to obtain energy in various types of conditions. Another principal attribute of *C. violaceum*, when compared with other sequenced microorganisms, is its high percentage of ORFs (5.8%) related to cell motility and secretion (COG category N) described until now (Vasconcelos et al., 2003; Pereira et al., 2004). Genes related to chemotaxis and flagella are typical adaptations of soil and water dwelling bacteria. A large number of transport-related proteins (496 ORFs, 11.2% of the valid ORFs) are associated with metabolic transport, multidrug-resistance proteins, membrane receptors and porins, suggesting intensive interactions with the different habitats (Vasconcelos et al., 2003).

Additionally, *C. violaceum* has several ORFs related to tolerance against antimicrobial compounds (CV0700), solvents and heavy metals, which results in greater competitiveness compared to other microorganisms under the same conditions. All these features, taken together, are strong attributes to enhance its ability to survive in a wide range of soil and water environments, most of them depleted of nutrients and energy sources (Walker, 1990).

IRON METABOLISM

Synthesis of iron-chelating compounds

All microorganisms need iron, the availability of which is limited in most aerobic environments, since it is present only in insoluble mineral complexes or bound to mammalian or plant host's iron-binding proteins. Therefore, many microorganisms have developed efficient ways to obtain iron under deprived conditions. One important mechanism is gene clusters encoding enzymes or receptors that recognize low molecular weight iron-chelating compounds, called siderophores. These siderophores are secreted into the environment, where they bind ferric iron with high affinity in a mechanism recognized as crucial to increase competitiveness and adaptation to different environmental conditions (Lankford, 1973; Crosa, 1989; Payne, 1994).

Enterobactins are classic examples of iron-chelating compounds involved in iron transportation from the environment into the bacterium cytoplasm. In the biosynthetic pathway of enterobactin, EntD is a key protein in the final conversion of 2,3-dihydroxybenzoic acid and L-serine into enterobactin. Studies involving EntD$^-$ and EntB$^-$ mutants of *E. coli* have shown that these genes are essential for bacterial growth in iron-deficient environments (Armstrong et al., 1989; Coderre and Earhart, 1989; Liu et al., 1989; Nahlik et al., 1989; Hantash and Earhart, 2000). ORFs related to the synthesis of enterobactin; *entA* (CV1482), *entB* (CV1483), *entC* (CV1485), *entE* (CV1484), *entD* (CV2650), *entF* (CV1486, CV2233) have been identified in

C. violaceum. Similar iron-chelating compounds have been described in other microorganisms, e.g., vibriobactin in *Vibrio cholerae* (Wyckoff et al., 1997), pyochelin and pyoverdine in *Pseudomonas* spp. (Poole and McKay, 2003), and myxochelin in myxobacteria (Silakowski et al., 2000).

In Gram-negative bacteria, outer membrane receptors, such as FepA in *E. coli* and ViuA in *V. cholerae* bind the ferrisiderophore complex across the inner membrane (Klebba et al., 1993; Wyckoff et al., 1997). A second receptor complex is needed, the major component of which is TonB, a 27-kDa protein that facilitates energy transfer from the proton motive force to outer receptors. B-12 and colicin receptors also make use of the TonB system to drive active transport in the outer membrane. In *E. coli*, the TonB protein interacts with outer membrane receptor proteins that carry out high-affinity binding and energy-dependent uptake of specific substrates into the periplasmic space. In the absence of TonB, these receptors bind to their substrates but do not carry out active transport (Klebba et al., 1993; Postle, 1993). The proteins that are currently known to interact with TonB include the ones encoded by the genes *btuB*, *cirA*, *fatA*, *fcuT*, *fecA*, *fhuE*, *fptA*, *hemR*, *irgA*, *iutA*, *pfeA*, *pupA*, and *tbp1*. Outer membrane receptors *fepA* (CV2230), *fepB* (CV2239), *fepC* (CV2234), *fepG* (CV2235), *fepD* (CV2236), and components of a second receptor complex that drive active transport in the outer membrane, with TonB-dependent receptors (CV0077, CV1019, CV1699, CV1970, CV1982, CV3188, CV3896, CV4254) were found in the *C. violaceum* genome. Furthermore, colicins *exbB* (CV0399), $exbB_1$ (CV1972), $exbB_2$ (CV1984), $exbD_1$ (CV1985), $exbD_2$ (CV0398), $exbD_3$ (CV1974), $exbD_4$ (CV1986), and $exbD_5$ (CV1973) were detected. Genes related to enterochelin esterase (*fes*, CV2231), iron-chelator protein (*mxcB*, CV2466) and ferrisiderophore reductase C (*ubiB*, CV3784) were also found in this genome.

Besides being crucial for the adaptability and competitiveness of *C. violaceum*, the iron-related genes may also play an important role in the virulence and infectious ability of the microorganisms. Some pathogenic bacteria express surface receptors to capture eukaryotic iron-binding compounds, while others have evolved siderophores to scavenge iron from iron-binding host proteins (Takase et al., 2000). Classic examples are the enterobactin/enterochelin gene clusters found in *E. coli* and *Salmonella* spp., with similar moieties in other pathogens (Liu et al., 1989; Nahlik et al., 1989; Poole and McKay, 2003). Further details about genes of *C. violaceum* related to pathogenicity can be found in Brito et al. (2004).

Synthesis of bacterioferritin, and other mechanisms related to iron uptake

Iron-depletion can be ameliorated through the use of storage proteins. Bacteria have two types of iron-storage proteins, the haem-containing bacterioferritins (BFR) and the haem-free ferritins. In *E. coli*, BFR (also known as cytochrome b1 or cytochrome b557) consists of 24 identical subunits that pack together to form a highly symmetrical, nearly spherical shell surrounding a central cavity. Studies using X-ray crystallography have revealed a close structural similarity between BFR and the ferritins, a family of iron-storage proteins found in both eukaryota and prokaryota. Some bacteria contain two BFR subunits, or two ferritin subunits that in most cases co-assemble. Others possess both BFR and ferritin, while some appear to lack any type of iron-storage protein; the reasons for these differences are unknown. Studies with mutants have shown that ferritin enhances growth during iron starvation and accumulates iron in the stationary growth phase (Andrews, 1998). Two ORFs related to BFR (CV3552 and

CV3399) and one probable BFR co-migratory oxidoreductase protein (CV2815) have been detected in *C. violaceum*.

Another mechanism of iron uptake detected in *C. violaceum* is related to ferric citrate transport system permease protein (*fecC* CV1909, *fecD* CV1488, CV1794, *fecE* CV3899), which facilitates iron diffusion in high concentration of this nutrient. *Escherichia coli* also has an iron (II) transport system (*feo*), which may make an important contribution to the iron supply of the cell under anaerobic conditions; consequently the transport of ferrous iron may be ATP dependent (Kammler et al., 1993). *feoB* was detected (CV3553) in *C. violaceum*, as well as other ORFs, such as *fhuA* (CV2251) and *fhuC* (CV1560, 1487), both involved in an active transport against a concentration gradient with the use of metabolic energy inputs.

Fifty ORFs are related to iron metabolism in *C. violaceum*, a considerable number in comparison to other bacterial genomes that have been sequenced, probably indicating a major role for survival under various environmental conditions.

CONTROL OF CELL OSMOTIC PRESSURE AND PH

Osmotic movement of water across bacterial cell membranes is an extremely important mechanism to maintain cell turgor. Proteins related to adaptation to differences in osmotic pressure in the environment are therefore crucial for cell survival and affect their adaptability. Osmotically inducible proteins were detected in *C. violaceum*, with high similarity to organic hydroperoxide resistance protein (CV0209, CV2493). However, an ORF showing similarity (6e-16) with the osmotically inducible lipoprotein OsmB (CV3209) was found, but none was found similar to OsmC. In *E. coli*, the expression of the *osmB* gene is regulated at the transcriptional level by two promoters that respond to the growth phase and to hyperosmolarity conditions, *osmB*p1 and *osmB*p2. The transcription of *osmB* from *osmB*p2 is induced by elevated osmolarity, or upon reaching the stationary phase, and transcription from *osmB*p1 occurs when both osmotic and growth phase signals are present (Jung et al., 1990).

Transport of K^+ ions, as well as increases in the intracellular concentration of osmoprotectans, such as glutamate, proline, threhalose and glycine, are also important for bacterial survival under osmotic pressure stress (Jung et al., 1990; Doyle et al., 1998). Examples in *C. violaceum* include the *kup1* (CV2731) and *kup2* (CV0573) genes (low-affinity potassium transport system), *kdpABC* (CV1599, 1598, 1597), the *kdpDE* (CV1596, 1595) operons (high-affinity potassium transport system), the *kefB* (CV3326) gene (glutathione-regulated potassium-efflux system K^+/H^+ antiporter transmembrane protein), the inward rectifier potassium channel transmembrane protein (CV1109), genes related to sodium chloride (CV1670, CV2040, CV2223, CV2225, CV2380, CV3281, CV3650, CV4369), sodium-glutamate symporter *gltS* (CV1105) and *gltP* (CV1198, CV3409), and proline transports *proP* (CV1299, CV2901), *proV* (CV1197) and *proY* (CV1138). ORFs with functions similar to a sodium/hydrogen exchanger (CV2903), and to a proton-dependent peptide transporter (CV3755), were found. These genes may act to control internal pH, extruding the H^+ generated during metabolism.

The porins form water-filled channels, which allow the diffusion of hydrophilic molecules into the periplasm, including large antibiotic molecules (Nikaido, 1994). In *E. coli*, the transcription of outer membrane porin genes *ompC* and *ompF* is regulated in response to medium osmolarity, by a two-component regulatory system, EnvZ, which is a transmembrane sensor, and OmpR, the response regulator (Jung et al., 1990; Cai and Inouye, 2002; Qin et al., 2003). In

C. violaceum, the outer membrane protein OmpC (CV3424) and the proteins of the regulatory system, EnvZ (CV0217) and OmpR (CV0216, CV3107), were found.

Another interesting gene annotated in *C. violaceum* was aquaporin Z (*aqpZ*, CV2864). The aquaporins are transmembrane water-channel proteins present in plants and animals (Chrispeels and Agre, 1994). These channels are water selective and do not allow ions or metabolites to pass through them, regulating osmotically driven movement of water in both directions, maintaining cell turgor during volume expansion in rapidly growing cells. The importance of these channels is due to their mediation of rapid entry or exit of water in response to abrupt changes in environmental osmolarity. In bacteria, the first sequence homologous to aquaporin was *glpF*, which encodes a glycerol facilitator that displays minimal water permeability, and the *aqpZ* sequence of *E. coli* encodes a polypeptide with 28-38% identity to known aquaporins (Heller et al., 1980; Calamita et al., 1995). The *C. violaceum aqpZ* gene shows 79-86% of similarity with that of *P. aeruginosa*. An operon containing *glpF*, *glpD*, *glpT*, *glpK*, and *glpR* (CV0252, 0254, 0253, 0251, 0136) genes was also found in *C. violaceum*. The expression of *aqpZ* conferred a 15-fold increase in osmotic water permeability in *Xenopus* oocytes, but it failed to transport nonionic solutes, such as urea and glycerol. In contrast, *Xenopus* oocytes expressing *glpF* transported glycerol, but they had limited osmotic water permeability. A phylogenetic comparison of aquaporins revealed a large difference between *aqpZ* and *glpF*, consistent with an ancient gene divergence (Preston et al., 1992). AqpZ-like proteins seem also to be necessary for virulence in some pathogenic bacteria (Calamita et al., 1995, 1998; Fushimi et al., 1997; Calamita, 2000).

OXIDATIVE STRESS

The advent of oxygen in the atmosphere was one of the first major pollution events on earth. The reaction between ferrous iron, very abundant in the reductive early atmosphere, and oxygen results in the formation of reactive oxygen species (ROS), which can damage proteins, DNA and lipids. The ROS include hydrogen peroxide (H_2O_2), the superoxide anion (O_2^-), the hydroxyl radical (OH), and organic hydroperoxides (ROOH) (Beckman and Ames, 1998). Bacteria have numerous enzymes to detoxify ROS, such as catalases, superoxide dismutases, alkyl hydroperoxide reductase, and related peroxidases of the AhpC/thiol-specific antioxidant family (Fuangthong et al., 2001; Fuangthong and Helmann, 2002). In *E coli*, the common responses to oxidative stress are controlled by two major transcriptional regulators, SoxRS and OxyR, which induce the expression of antioxidant activities in response to O_2^-/H_2O_2 and H_2O_2 stress, respectively (Storz and Imlay, 1999; Manchado et al., 2000; Pomposiello and Demple, 2001).

The *soxRS* regulon is induced in a two-stage process (Nunoshiba et al., 1992). Superoxide-generating compounds, such as paraquat or H_2O_2, activate the transcription factor SoxR by the univalent oxidation of the [2Fe-2S] clusters of the protein. Oxidized SoxR induces the expression of a second transcription factor, SoxS, which in turn activates the transcription of a set of genes in this regulon. Among the SoxRS-regulated genes are *micF* (antisense RNA to the porin OmpF), *sodA* (manganese superoxide dismutase), *inaA* (pH-inducible protein involved in stress response), *fumC* (heat-resistant fumarase), and *fpr* (NADPH-ferredoxin reductase). Evidence for several pathways of SoxR activation, mediated by modifications of [2Fe-2S] centers, has emerged from recent data. The direct oxidation of [2Fe-2S] involves any event that may interfere with the pathway maintaining SoxR in a reduced inactive form; hydrogen perox-

ide and direct nitrosylation by NO can trigger SoxR activation. Recently, it was reported that although it is not involved in genetic regulation, SoxRS decreases the mortality of cells due to ozone (Jimenez-Arribas et al., 2001). The multiple possibilities for SoxR activation, along with signal amplification via the two-stage process, constitute a unique and sensitive system, enabling cells to rapidly induce a protective response to a broad range of environmental changes, including oxidative stress (Richards et al., 1998; Cabiscol et al., 2000; Manchado et al., 2000; Touati, 2000; Jimenez-Arribas et al., 2001; Michán et al., 2002). In *C. violaceum*, *soxR* (CV2793) is present, but not the genes *soxS*, *micF*, *inaA*, and *sodA*. However, *soxS* is also lacking in other genomes, including *Agrobacterium tumefaciens*, *Bacillus halodurans*, *Pseudomonas putida*, *Xanthomonas axopodis* pv. *citri*, *X. campestris* pv. *campestris*, *Mesorhizobium loti*, *Sinorhizobium meliloti*, and *V. choleare*. Recent research indicated that SoxR and SoxS might bind to different regions within the *fumC* promoter, or an unknown intermediate might be acting in the transcription of *fumC*. Thus, the regulatory role of SoxR may be more complex than what was previously known from *E. coli* (Fuentes et al., 2001). Besides playing an important role in adaptability to different environments, resistance to oxidative stress may contribute to the virulence of pathogenic bacteria. Indeed, in *Erwinia chrysanthemi*, SoxR interacted with *suf* genes in a virulence response (Nachin et al., 2001).

In *E. coli,* the OxyR transcription factor is activated by hydrogen peroxide through the formation of an intramolecular disulfide bond (Choi et al., 2001), which in turn can activate the expression of hydroperoxidase I (KatG), alkyl hydroperoxide reductase (AhpCF), DNA-binding protein (Dps), and other resistance proteins (Storz and Imlay, 1999). OxyR (CV3378) and the oxidative stress protein Dps (CV4253) were also identified in *C. violaceum*.

Another biochemical mechanism to sense peroxidative stress has been described in *B. subtilis*; it is triggered by OhrR, a member of a conserved family of organic peroxide-sensing transcription factors. OhrR represses transcription of the *ohrA* resistance gene by cooperative binding to two inverted repeat elements that overlap the promoter. Induction by peroxides requires a conserved Cys residue that is reversibly oxidized by peroxides to a sulfenic acid (Sukchawalit et al., 2001; Fuangthong et al., 2001). *C. violaceum* has an operon composed of *ohrA-ohrR* ORFs (CV0209, 0210). Other ORFs in *C. violaceum* that might be related to oxidative stress are those coding the hydroperoxide resistance protein OhrB (CV2493) and the MutT/nudix (nucleoside diphosphate linked to some other moeity X) family proteins (CV0032, CV1112, CV1586, CV1767, CV3401). The MutT homologues (MutT/MTH) remove oxidized nucleotide precursors so that they cannot be incorporated into DNA during replication; they are found in a variety of prokaryotic, viral and eukaryotic organisms (Lu et al., 2001). DsbA is the major disulfide oxidase in the bacterial periplasm (Goecke et al., 2002), and it is also present in *C. violaceum* (CV3998).

ORFs related to *aidB* (acyl-coA dehydrogenase) were detected in *C. violaceum* (CV1785, CV2084, CV2723, CV3816, CV4136, CV4139). In *E. coli*, Ada protein activates σ^{70}-dependent transcription at three different promoters (*ada*, *aidB*, and *alkA*). The *aidB* gene is not expressed constitutively, but its transcription is induced via distinct mechanisms in response to: i) alkylating agents; ii) acetate at a slightly acidic pH, and iii) anoxia. Induction by alkylating agents is mediated by the transcriptional activator Ada, in its methylated form (meAda); the other forms of induction are Ada-independent and require sigma-S, the alternative sigma factor mainly expressed during the stationary phase of bacterial growth. The leucine-responsive protein (Lrp), a global regulatory protein in *E. coli* that activates expression of more than a

dozen operons and represses expression of another dozen (Chen et al., 2001), is also related to the regulation of those genes (Landini et al., 1996; Landini and Busby, 1999).

Several glutathione-S-transferases (CV0194, CV0289, CV0905, CV0972, CV1086, CV1164, CV1775, CV2424, CV2745, CV3024, CV3306, CV4373) and glutathione peroxides (CV1107, CV3555, CV3787) that are related to protection mechanisms are also present in *C. violaceum*, and they may play a crucial role in adaptability. Bacterial GSTs of known function often have a specific, growth-supporting role in biodegradative metabolism. Some regulatory proteins, such as the stringent starvation proteins, also belong to the GST family (Vuilleumier, 1997). In eukaryotes, GSTs participate in the detoxification of reactive electrophilic compounds by catalyzing their conjugation to glutathione. Among other genes involved in oxidative stress, genes encoding GSTs have been induced by aluminum (Al) stress in *Arabidopsis* (Richards et al., 1998), and tobacco (Ezaki et al., 1995).

PROTEINS RELATED TO TOLERANCE TO TEMPERATURE, ACID AND UV LIGHT STRESSES

Optimum temperature for growth of *C. violaceum* ranges from 30 to 37°C, with a minimum of 1 to 15°C and a maximum of 40°C, but 20% of the strains can grow at 44°C (Sneath, 1984), and it has been isolated in Antarctica (Kriss et al., 1976). Marine teleosts from polar oceans can be protected from freezing in icy seawater by anti-freezing proteins or glycoproteins that act by binding to the ice crystals within the fish, preventing the growth of the crystals. A model has been proposed in which the protein binds to an ice nucleation structure, in a zipper-like fashion, via hydrogen bonding of threonine side chains (with an 11-residue period) to oxygen atoms in the ice lattice. The growth of ice crystals is thus stopped, or retarded, and the freezing point depressed (Chou, 1992; Chen et al., 1997). Thirty-nine ORFs showing anti-freezing domain proteins were detected in *C. violaceum*; these could play a role in mechanisms that allow the bacteria to survive low temperatures, or other kinds of stress, such as acid conditions or high temperature. Several genes related to heat shock proteins were also found in *C. violaceum*, including an operon of the DnaJ-DnaK-GrpE system (CV1645, 1643, 1642), the GroEL/GroES system (*mopA* and *mopB*, CV3233, 3232, CV4014, 4015), sigma factors (CV0585, CV2058, CV3332), the chaperonin ClpA/B system (CV3965), HscA/B co-chaperone (CV1089, CV1091), HtpG (CV1318), HtpX (CV3109, CV4263), HslO (CV2000), HslU (CV0402), and HslV (CV0401). These proteins may also be involved in adaptability to a wide range of temperatures.

Although *C. violaceum* is described as being unable to grow below pH 5.0 (Sneath, 1984), the pH of waters of the Negro River ranges from 3.8 to 4.9 (Walker, 1990). Thus, genes such as the acid shock (*asr*) (CV4241) gene, which has a high similarity with the same gene in *Enterobacter cloacae*, may play an important role in survival under these conditions. Furthermore, as most Brazilian soils are acidic and this is often associated with Al toxicity, an Al resistance protein (CV3507) could have a protective role against the toxic levels of Al. This ORF is similar to the one found in *Neisseria meningitidis* (4e-54) and *Bacillus halodurans* (3e-52), to a protein associated with Al tolerance in *Arthrobacter viscosus* (1e-46) (Jo et al., 1997), and to a PP-loop superfamily ATPase that confers Al resistance in *Clostridium acetobutylicum* (Nolling et al., 2001). Heat shock proteins may also have protective activity under Al stress (Ezaki et al., 1998).

An efficient mechanism against UV light was found in the *C. violaceum* genome, including two copies of *uvrA*, *uvrB*, *uvrC* and *uvrD* genes (CV1893, CV3152, CV1305), and one copy of *uvrD* (CV0205). Additionally, the well-studied violacein operon (CV3274, 3273, 3272, 3271) (August et al., 2000) protects *C. violaceum* from radiation (Caldas et al., 1978).

STARVATION GENES

Several genes that promote survival under starvation conditions were detected in *C. violaceum*, some of them also being related to protection against oxidative damage. A DNA-binding stress protein (Dps, CV4253), already described in item Oxidative stress, is similar to the one found in *Listeria innocua* (Bozzi et al., 1997; Chiancone, 1997). In *E. coli*, Dps is synthesized during prolonged starvation in order to protect DNA from oxidative damage and from other stress conditions during the stationary phase. Studies performed *in vitro* showed that Dps forms extremely stable complexes with DNA, without apparent sequence specificity, while mutant cells lacking Dps develop dramatic changes in the pattern of proteins synthesized during starvation (Almiron et al, 1992).

Two carbon starvation genes *cstA* and *cstB* were described in *E. coli*; it was suggested that *cstA* was involved in peptide utilization (Schultz and Matin, 1991). In *C. violaceum* only *cstA* was detected (CV1662, CV0762), as in *P. aeruginosa*. Stringent starvation proteins SspA (CV4005) and SspB (CV4004) were present in *C. violaceum*. In *E. coli* these proteins are synthesized for survival during growth and prolonged starvation conditions, being induced by glucose, nitrogen, phosphate or amino acid starvation (Williams et al., 1994).

The stationary-phase survival protein (SurE, CV3679) has an important role in stationary-phase survival; *E. coli* mutants lacking this protein survived poorly under high temperatures and salt concentrations (Li et al., 1994). In *E. coli* and *P. aeruginosa*, *surE* is co-transcribed with *pcm*, a gene encoding the L-isoaspartyl protein repair methyltransferase, which probably plays a role in the repair and/or degradation of spontaneously damaged proteins. There is evidence of an interaction between *pcm* and *surE* to avoid protein damage (Fu et al., 1991; Li et al., 1994; Visick et al., 1998). Two ORFs similar to *pcm* (CV3680, CV0234) were detected in *C. violaceum*. The first one is apparently responsible for the co-transcription with *surE*, while the second could be related to co-transcription with DNA repair genes, since CV3680 is localized within the *glp* operon (CV0251, 0252, 0253, 0254), associated with cell protection against damage. Another survival protein precursor, a peptidyl-prolyl cis-trans isomerase (SurA, CV4230) was detected in *C. violaceum*. These molecular chaperones are required in the folding processes of proteins that are necessary to cross the plasma membrane and to be released into the periplasm in Gram-negative bacteria. The ORF CV2816 is probably related to PhoH, a cytoplasmic protein, and a predicted ATPase that is induced by phosphorus starvation.

OTHER CELL PROTECTION STRATEGIES

Other proteins involved in cell protection are present in *C. violaceum* (CV1796), such as OmlA, an outer membrane lipoprotein proposed to have the structural role of maintaining the cell envelope integrity under stress conditions (Oschsner et al., 1999). In *P. aeruginosa*, *fur* (ferric uptake regulation protein) overlaps with *omlA* (Oschner et al., 1999) and in *Burkholderia* both genes are co-localized, although in the latter bacterium the role of *fur* in iron uptake is not

clear (Lowe et al., 2001). Similarly, in *C. violaceum*, the *fur* (CV1797) and *omlA* (CV1796) ORFs are adjacent.

Genes that help protect against cell desiccation were also annotated in the genome of *C. violaceum*, including *manA* (mannose-6-phosphate isomerase, CV2312), *manC* (mannose-1-phosphate guanylyltransferase, CV3178), *ugd* (UDP-glucose-6-dehydrogense, CV3041), *galE* (UDP-galactose-4-epimerase, CV3884) and *galU* (glucose-1-phosphate uridylyltransferase, CV3901). Another interesting gene detected in *C. violaceum* is *mscL* (CV1360). Although its function is still not well understood, similar proteins are found in several bacteria; in *E. coli mscL* encodes a channel that is opened by membrane stretch force, probably serving as an osmotic gauge, therefore transducing physical stresses at the cell membrane into an eletromagnetical response (Moe et al., 1998).

The universal stress proteins are small cytoplasmic proteins whose expression is enhanced several-fold when cellular viability is challenged with heat shock, nutrient starvation, stress agents that arrest cell growth, or DNA-damaging agents. In *E. coli* these genes are induced by the stationary phase and by a variety of stresses causing growth arrest of cells, and they are not dependent on *rpoS*. Also in *E. coli*, Usp-mutants were found to be sensitive to UV light (Gustavsson and Nystrom, 2002). Genes encoding putative universal stress proteins were detected in *C. violaceum* (CV3769, CV4160, CV4192, CV4193); they might protect the bacterium from normal stress factors, such as exposure to UV light in aquatic environments.

CONCLUSIONS

Chromobacterium violaceum occurs abundantly in water environments in the Amazon region, as well as in soils and water of other tropical and subtropical regions. Most of those ecosystems are very poor on nutrients, and organisms are frequently submitted to environmental stresses, such as high temperature, UV exposure, and acidity. Several mechanisms that might be related to the remarkable adaptability of this bacterium were revealed in the complete sequencing of its genome. Alternative pathways for energy generation, an impressive number of proteins related to transport, cell motility, and secretion, many proteins related to iron metabolism, osmotically inducible proteins, membrane porins, proteins related to tolerance against temperature, acid and UV stresses, among others, help explain its extraordinary competitiveness and tolerance to stress.

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