Drug resistance in *Chromobacterium violaceum*

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ABSTRACT. *Chromobacterium violaceum* is a free-living bacterium commonly found in aquatic habitats of tropical and subtropical regions of the world. This bacterium is able to produce a large variety of products of biotechnological and pharmacological use. Although *C. violaceum* is considered to be non-pathogenic, some cases of severe infections in humans and other animals have been reported. Genomic data on the

type strain ATCC 12472[T] has provided a comprehensive basis for detailed studies of pathogenicity, virulence and drug resistance genes. A large number of open reading frames associated with various mechanisms of drug resistance were found, comprising a remarkable feature of this organism. Amongst these, beta-lactam (penicillin and cephalosporin) and multidrug resistance genes (drug efflux pumps) were the most numerous. In addition, genes associated with bacitracin, bicyclomycin, chloramphenicol, kasugamycin, and methylenomycin were also found. It is postulated that these genes contribute to the ability of *C. violaceum* to compete with other bacteria in the environment, and also may help to explain the common drug resistance phenotypes observed in infections caused by this bacterium.

Key words: Antibiotics, Resistance, *Chromobacterium violaceum*

INTRODUCTION

Antibiotics are characterized by their chemical composition and mode of action in the target organism. The main targets for action in bacteria include the prokaryotic cell wall, the cell membrane, protein synthesis, enzymatic pathways, and DNA replication (Brooks et al., 2001). There are many different mechanisms by which microorganisms can express resistance to antibiotics, and some are very well documented: 1) they may produce enzymes that destroy the active drug, 2) they can change membrane permeability to block entry or express transport systems that actively pump the drug out into the extracellular environment, 3) they express altered structural targets, 4) they express metabolic pathways that bypass the reaction inhibited by the drug, or 5) they express altered enzymes that are less affected by the drug (Salyers and Whitt, 2001).

The origin of drug resistance may be genetic or non-genetic. Active replication of bacteria is usually required for the action of most antibacterial drugs. Consequently, microorganisms that are metabolically inactive (non-multiplying or sporulated) may be phenotypically resistant to drugs. Also, microorganisms may lose the specific structural drug target for several generations, and thus become temporarily resistant. These non-genetic mechanisms of drug resistance (Brooks et al., 2001), though important in many circumstances, are temporary reversible phenotypical states. However, drug resistance that emerges as a result of genetic change and subsequent selection by antimicrobial drugs, and thus may become permanent in the bacterium and be passed onto its next generations, comprises one of the greatest problems in human and veterinary medical care (Shao et al., 2002; Perera et al., 2003), and can scale up into multidrug resistance problems (Martinez et al., 2000).

Antibiotic resistance can be mediated by chromosomal and extrachromosomal genes, which can be transferred to other cells by the general mechanisms of genetic recombination, such as transduction, transformation, conjugation, and transposition (Salyers and Whitt, 2001). Some recent data suggest that different species of bacteria can develop a "mutator phenotype" under stress conditions, favoring the production of mutations associated with drug resistance (Oliver et al., 2000). In some bacteria, the expression of antibiotic production pathways, and

their drug resistance counterparts, are under the control of signal sensing systems, such as quorum sensing, in which production is dependent upon the cell cycle stage, on concentrations of signals associated with high population density or on factors excreted by competing bacteria (Strauss, 1999).

Chromobacterium violaceum is a Gram-negative bacillus that can be isolated from aquatic habitats in tropical and sub-tropical regions (Richard, 1993). It can sometimes cause life-threatening infections in humans and other animals, commonly sepsis, followed by cutaneous involvement and liver abscesses, though diarrheal episodes have also been reported (Liu et al., 1989; Richard, 1993; Ti et al., 1993; Chong and Lam, 1997; Midani and Rathore, 1998; Martinez et al., 2000; Dromigny et al., 2002). These are relatively rare occurrences, but some cases of septicemic diseases have been reported in South America (Petrillo et al., 1984; Kaufman et al., 1986; Martinez et al., 2000).

In fact, pathogenicity islands and other virulence genes were identified in the *C. violaceum* genome, demonstrating that this bacterium does have a genetic potential for being pathogenic (Vasconcelos et al., 2003). Another remarkable characteristic of *C. violaceum* is the resistance to a relatively broad range of antibiotics (rifampin, vancomycin, ampicilin, cephalosporins), a phenomenon that makes the treatment of infections difficult (Aldridge et al., 1988; Berkowitz and Metchock, 1995; Martinez et al., 2000).

The completion of genomic sequencing of *C. violaceum* (Vasconcelos et al., 2003) created a sound basis for studying antibiotic resistance under a new perspective, that of comparative genomics. Herein, we describe the results of a comprehensive study of *C. violaceum* ATCC 12472[T], focusing upon genes associated with drug resistance identified in the genome. Open reading frame (ORF) similarity analyses revealed a large number of sequences in *C. violaceum* ATCC 12472[T] related to known antibiotic resistance genes. These are related to beta-lactam (Table 1), multidrug (Table 2) and other resistance genes (Table 3).

Table 1. *Chromobacterium violaceum* OFRs related with beta-lactam antibiotics.

ORF	Gene	Product	BLAST analysis results
CV0834	*mrcA*	penicillin-binding protein 1A (penicillin-insensitive transglycosylase/penicillin-sensitive transpeptidase)	*Ralstonia solanacearum* Score = 113 bits (57) Expect = 5e-23 Identities = 159/193 (82%)
CV0902		D-alanyl-D-alanine dipeptidase	*Streptomyces coelicolor* Score = 64.1 bits (33) Expect = 2e-07 Identities = 55/66 (83%)
CV1125	*dacB*	serine-type D-Ala-D-Ala carboxypeptidase	*Pseudomonas aeruginosa* Score = 235 bits (600) Expect = 6e-61 Identities = 151/418 (36%)
CV1310	*ampC*	beta-lactamase precursor (cephalosporinase)	*P. aeruginosa* Score = 81.8 bits (41) Expect = 9e-14 Identities = 98/117 (83%)

Table 1. Continued.

ORF	Gene	Product	BLAST analysis results
CV1341		probable metal-dependent hydrolases of the beta-lactamase superfamily II	*Methanosarcina acetivorans* Score = 174 bits (442) Expect = 7e-43 Identities = 102/258 (39%)
CV2562	*pbpB*	penicillin-binding protein 2	*R. metallidurans* Score = 162 bits (84) Expect = 2e-36 Identities = 126/147 (85%)
CV2808	*ampH*	penicillin-binding protein ampH	*Yersinia pestis* Score = 355 bits (912) Expect = 4e-97 Identities = 187/341 (54%)
CV3094	*dacC*	D-ala-D-ala-carboxypeptidase	*P. aeruginosa* Score = 89.7 bits (45) Expect = 4e-16 Identities = 72/81 (88%)
CV3150		probable beta-lactamase precursor	*R. solanacearum* Score = 78.2 bits (191) Expect = 8e-14 Identities = 63/201 (31%)
CV3153	*pbpG*	D-alanyl-D-alanine-endopeptidase	*R. solanacearum* Score = 60.0 bits (30) Expect = 2e-07 Identities = 69/82 (84%)
CV3354	*prc*	carboxy-terminal processing protease	*P. aeruginosa* Score = 91.7 bits (46) Expect = 1e-16 Identities = 151/186 (81%)
CV4349	*fts*I	probable penicillin-binding 3 precursor	*R. solanacearum* Score = 75.8 bits (38) Expect = 8e-12 Identities = 77/90 (85%)
CV4360	*mrdA*	penicillin-binding protein 2 (PBP-2)	*R. solanacearum* Score = 81.8 bits (41) Expect = 2e-13 Identities = 86/101 (85%)

ORF = open reading frame determined in the *Chromobacterium violaceum* genome (Vasconcelos et al., 2003).

Table 2. OFRs related to multidrug resistance.

ORF	Gene	Product	BLAST analysis results
CV0020	*bcr*	bicyclomycin resistance protein (sulfonamide resistance protein)	*Pseudomonas aeruginosa* Score = 110 bits (274) Expect = 3e-23 Identities = 78/246 (31%)

Table 2. Continued.

ORF	Gene	Product	BLAST analysis results
CV0122		probable multidrug resistance protein	*Yersinia pestis* Score = 199 bits (506) Expect = 4e-50 Identities = 123/375 (32%)
CV0434	*acrD*	acriflavin resistance protein D	*P. aeruginosa* Score = 161 bits (81) Expect = 3e-37 Identities = 182/213 (85%)
CV0435	*emrB*	multidrug resistance protein B	*Ralstonia solanacearum* Score = 300 bits (156) Expect = 3e-78 Identities = 468/624 (75%)
CV0436	*acrR*	acrab operon repressor	*P. aeruginosa* Score = 65.9 bits (33) Expect = 3e-09 Identities = 51/57 (89%)
CV0631	*mdlA*	ABC transporter ATP-binding membrane protein	*P. aeruginosa* Score = 91.7 bits (46) Expect = 1e-16 Identities = 70/78 (89%)
CV0700		probable multidrug/chloramphenicol efflux transporter	*R. solanacearum* Score = 594 bits (309) Expect = e-167 Identities = 787/1016 (77%)
CV0708	*emrB*	multidrug resistance protein B	*R. solanacearum* Score = 300 bits (156) Expect = 3e-78 Identities = 468/624 (75%)
CV0709	*emrK*	multidrug resistance protein K	*R. solanacearum* Score = 294 bits (153) Expect = 1e-76 Identities = 385/501 (76%)
CV0710		probable outer membrane multidrug resistance protein	*R. solanacearum* Score = 85.7 bits (43) Expect = 7e-15 Identities = 106/127 (83%)
CV0766	*emrB*	multidrug resistance protein B	*P. aeruginosa* Score = 115 bits (58) Expect = 8e-24 Identities = 106/122 (86%)
CV0767	*emrA*	multidrug resistance protein A	*R. solanacearum* Score = 214 bits (108) Expect = 9e-54 Identities = 276/332 (83%)

Table 2. Continued.

ORF	Gene	Product	BLAST analysis results
CV0768		putative outer membrane multidrug resistance lipoprotein	*P. aeruginosa* Score = 71.8 bits (37) Expect = 2e-09 Identities = 49/55 (89%)
CV0769	*emrR*	transcriptional repressor emr operon, MarR family	*Yersinia pestis* Score = 131 bits (330) Expect = 3e-30 Identities = 72/159 (45%)
CV0771	*bcr*	bicyclomycin resistance protein (sulfonamide resistance protein)	*Xanthomonas campestris* Score = 64.1 bits (33) Expect = 4e-07 Identities = 117/159 (73%)
CV0798		fosmidomycin resistance protein - MFS family	*R. solanacearum* Score = 182 bits (92) Expect = 3e-44 Identities = 341/424 (80%)
CV1078	*emrY*	multidrug resistance transmembrane protein	*R. solanacearum* Score = 57.0 bits (136) Expect = 4e-07 Identities = 56/214 (26%)
CV1179		probable multidrug resistance protein	*Pseudomonas fluorescens* Score = 79.5 bits (41) Expect = 9e-12 Identities = 53/59 (89%)
CV1205	*cmr*	multidrug translocase mdfA (chloramphenicol resistance pump cmr)	*R. solanacearum* Score = 56.0 bits (28) Expect = 6e-06 Identities = 31/32 (96%)
CV1278		probable multidrug transmembrane resistance signal peptide protein	*Sinorhizobium meliloti* Score = 94.0 bits (232) Expect = 4e-19 Identities = 46/80 (57%)
CV1279		probable multidrug transmembrane resistance signal peptide protein	*S. meliloti* Score = 104 bits (259) Expect = 3e-22 Identities = 53/112 (47%)
CV1455		probable multiple antibiotic resistance protein MarC	*Neisseria meningitides* Score = 79.5 bits (41) Expect = 5e-12 Identities = 79/95 (81%)
CV1510		probable multidrug resistance protein	*Vibrio vulnificus* Score = 226 bits (575) Expect = 4e-58 Identities = 131/334 (39%)

Continued on next page

Table 2. Continued.

ORF	Gene	Product	BLAST analysis results
CV1593		probable transmembrane efflux protein/ multidrug resistance protein	*P. aeruginosa* Score = 102 bits (53) Expect = 1e-18 Identities = 87/104 (83%)
CV1626		putative multidrug resistance protein *acrB*, acriflavin resistance protein B	*P. aeruginosa* Score = 127 bits (64) Expect = 4e-27 Identities = 112/128 (87%)
CV1627	*acrE*	acriflavin resistance protein E precursor; probable RND efflux membrane fusion protein precursor	*Bradyrhizobium japonicum* Score = 283 bits (723) Expect = 2e-75 Identities = 153/374 (40%)
CV1769		probable resistance protein	*E. coli* Score = 187 bits (97) Expect = 4e-44 Identities = 355/484 (73%)
CV2240		probable multidrug efflux protein	*R. solanacearum* Score = 58.0 bits (29) Expect = 1e-06 Identities = 32/33 (96%)
CV2241	*acrB*	acriflavin resistance protein B	*P. aeruginosa* Score = 190 bits (96) Expect = 4e-46 Identities = 177/204 (86%)
CV2680	*emrE*	emrE protein (methyl viologen resistance protein C; ethidium resistance protein)	*Brucella melitensis* Score = 69.9 bits (36) Expect = 2e-09 Identities = 100/132 (75%)
CV2729		probable multiple antibiotic resistance protein MarC	*Caulobacter crescentus* Score = 153 bits (386) Expect = 2e-36 Identities = 91/230 (39%)
CV2848		probable outer membrane component of multidrug efflux pump	*Ralstonia metallidurans* Score = 133 bits (69) Expect = 7e-28 Identities = 223/300 (74%)
CV3243	*ebrB*	transmembrane multidrug resistance efflux protein	*X. axonopodis* Score = 129 bits (323) Expect = 8e-29 Identities = 69/162 (42%)
CV3736		probable multidrug resistance protein	*Streptomyces coelicolor* Score = 122 bits (306) Expect = 6e-27 Identities = 105/383 (27%)

Table 2. Continued.

ORF	Gene	Product	BLAST analysis results
CV3837		probable multidrug efflux membrane protein	*R. solanacearum* Score = 69.9 bits (36) Expect = 7e-09 Identities = 94/123 (76%)
CV3838		probable outer membrane drug efflux lipoprotein	*R. solanacearum* Score = 75.7 bits (39) Expect = 2e-10 Identities = 79/99 (79%)
CV3923		probable transport/efflux transmembrane protein	*R. solanacearum* Score = 66.1 bits (34) Expect = 9e-08 Identities = 88/115 (76%)
CV3924		probable transmembrane drug efflux protein	*R. solanacearum* Score = 192 bits (97) Expect = 9e-47 Identities = 232/277 (83%)
CV4317	*cmr*	multidrug translocase mdfA (chloramphenicol resistance pump cmr)	*P. putida* Score = 301 bits (771) Expect = 8e-81 Identities = 169/360 (46%)
CV4365		probable multiple antibiotic resistance protein MarC	*E. coli* Score = 144 bits (363) Expect = 7e-34 Identities = 85/199 (42%)

ORF = open reading frame determined in the *Chromobacterium violaceum* genome (Vasconcelos et al., 2003).

BETA-LACTAM ANTIBIOTIC (PENICILLIN AND CEPHALOSPORIN)-RESISTANCE GENES

All beta-lactam antibiotics are selective inhibitors of bacterial cell wall synthesis by interaction with the penicillin-biding proteins. After a beta-lactam antibiotic has attached to one or more penicillin receptors, the transpeptidation reaction is inhibited and peptidoglycan synthesis is blocked. This inhibition may be due to a structural similarity of these drugs to acyl-D-alanyl-D-alanine. The next step may involve inactivation of an inhibitor of autolytic enzymes in the cell wall. Resistance to this class of antibiotics can be due to the absence of some penicillin receptors (penicillin-biding proteins), and occurs as a result of chromosomal mutations, or from failure of the beta-lactam drug to activate the autolytic enzymes. Thus, the presence of penicillin-biding proteins *per se* is an essential characteristic of bacteria, and does not necessarily indicate resistance.

Genes that exhibit similarity to penicillin-binding proteins and to D-alanyl-D-alanine-endopeptidases of different bacterial species were identified in the *C. violaceum* genome (Table 1). These genes were expected, as they code for essential membrane proteins involved in the biosynthesis of murein and peptidoglycan, protein components of the cell wall. Sequence simi-

Table 3. ORFs related to miscellaneous resistance genes.

ORF	Gene	Product	BLAST analysis results
CV0121	*ksgA*	dimethyladenosine transferase	*Ralstonia metallidurans* Score = 133 bits (69) Expect = 4e-28 Identities = 197/261 (75%)
CV0719	*mmr*	methylenomycin A resistance protein	*Caulobacter crescentus* Score = 66.1 bits (34) Expect = 1e-07 Identities = 46/52 (88%)
CV1929	*bac*	bacitracin resistance protein (putative undecaprenol kinase)	*Magnetospirillum magnetotacticum* Score = 60.3 bits (31) Expect = 4e-06 Identities = 63/79 (79%)
CV2550		probable mitomycin resistance protein	*Xanthomonas axonopodis* Score = 86.3 bits (212) Expect = 7e-17 Identities = 39/79 (49%)

ORF = open reading frame determined in the *Chromobacterium violaceum* genome (Vasconcelos et al., 2003).

larities indicate that one domain of these proteins belongs to a large family of beta-lactam-recognizing proteins, which includes the active-site serine beta-lactamases (Pares et al., 1996).

Resistance to beta-lactams may also be determined by the organism's production of penicillin-destroying enzymes (beta-lactamases). Beta-lactamases open the beta-lactam ring of the penicillins and cephalosporins, destroying their antimicrobial activity. There are genes with similarity to beta-lactamases or precursor proteins in the genome of *C. violaceum* (Table 1), suggesting that this bacterium is able to produce such proteins.

D-Ala-D-Ala peptidase is a bacterial serine protease, a proteolytic enzyme with serine in its catalytic activity. These peptidases are ubiquitous, being found in viruses, bacteria and eukaryotes. They are involved in a wide range of peptidase activities, including exo-, endo-, oligo- and omega-peptidase activity. Over 20 families (denoted S1 to S27) of serine protease have been identified, being grouped into six classes (SA, SB, SC, SE, SF, and SG) on the basis of structural similarity and other functional features. Structures are known for classes SA, SB, SC, and SE. They appear to be unrelated, suggesting at least four evolutionary origins of serine peptidases, and possibly more.

D-Ala-D-Ala carboxypeptidases (S11 family) are involved in the metabolism of cell components. They are synthesized with a leader peptide that targets the protein to the cell membrane. After cleavage of the leader peptide, the enzyme is retained in the membrane by a C-terminal anchor. There are three families of serine-type D-Ala-D-Ala peptidases, which are also known as low-molecular weight penicillin-binding proteins (Rawlings and Barrett, 1994).

MULTIDRUG RESISTANCE SYSTEMS

Drug efflux is a major mechanism of multiple drug resistance in bacteria. Generally, this

is accomplished by efflux systems, responsible for unidirectional pumping of cytotoxic drugs to the extracellular environment. Although these efflux systems are usually chromosomally encoded, some may be present on plasmids. In addition to antibiotics, these pumps may export numerous dyes, detergents, disinfectants, inhibitors, organic solvents, and also homoserine lactones, involved in quorum sensing, a signaling system present in *C. violaceum* (Poole, 2001).

Five families of drug extrusion translocases have been identified based on sequence similarity. These are the MF (major facilitator), SMR (small multidrug resistance), RND (resistance nodulation cell division), ABC (ATP-binding cassette), and the recently identified MATE (multidrug and toxic compound extrusion) family (Nikaido, 1996; Brown et al., 1999).

The genome of *C. violaceum* contains genes with similarity to the *mar* (multiple antibiotic resistance) locus (Table 2). This locus is composed of the *mar*RAB operon, which controls multiple antibiotic resistance in *E. coli*. MarR (*sly*A) is a regulator, from the XylS/AraC family of transcriptional regulator proteins, whose product (MarR protein) alters the expression of several target genes. Structural studies showed MarR as a dimmer, with each subunit containing a winged-helix DNA binding motif (Alekshun et al., 2001). Numerous bacterial transcription regulatory proteins bind DNA via a helix-turn-helix motif. These proteins are very diverse, but for convenience may be grouped into subfamilies on the basis of sequence similarity. One such family, marR is grouped with a wide range of proteins, including emrR, hpcR, hpR, marR, pecS, petP, papX, prsX, ywaE, yxaD, and yybA. The Mar proteins are involved in multiple antibiotic resistance, a non-specific resistance system. The expression of the *mar* operon is controlled by a repressor, MarR, which interacts with a large number of compounds in order to induce transcription of the *mar* operon, possibly via loss of its DNA binding ability. *C. violaceum* also contains an ORF with similarity to *mar*C. Although the function of this gene is unknown, similarity with efflux protein genes of *E. coli* suggests the same activity for it.

Genes with similarity to the *emr*RAB locus of *E. coli* are also present in *C. violaceum* (Lomovskaya and Lewis, 1992). This operon encodes a multidrug resistance pump of the MF-family that protects the cell from several chemically unrelated antimicrobial agents, e.g., the protonophores carbonyl cyanide m-chlorophenylhydrazone (CCCP), tetrachlorosalicyl anilide and the antibiotics, nalidixic acid and thiolactomycin. The *emr*R is the first gene of the operon, and was shown to be a repressor of microcin biosynthesis (del Castillo et al., 1990). Overproduction of the EmrR protein (with a multicopy vector containing the cloned *emr*R gene) suppressed transcription of the *emr* operon. A mutation in the *emr*R gene led to over-expression of the EmrAB pump and increased resistance to antimicrobial agents. CCCP, nalidixic acid, and a number of other structurally unrelated chemicals induced expression of the *emr* genes, and the induction required an EmrR regulator. In conclusion, the *emr*RAB genes constitute an operon, and EmrR serves as its negative regulator. Some of the chemicals that induce the pump serve as its substrates, suggesting that their extrusion is the natural function of the pump (Lomovskaya et al., 1995).

Proteins of the SMR family are unusually small and are predicted to span the membrane four times. The *emr*E (*mvr*C) gene, the disruption of which causes hyper-susceptibility to tetraphenylphosphonium, methylviologen, and ethidium bromide, is a member of this family in *E. coli*. Overproduction of EmrE protein makes *E. coli* slightly more resistant to tetracycline, erythromycin, and sulfadiazine (Nikaido, 1996). *C. violaceum* contains a gene similar to *emr*E.

Some genes of *C. violaceum* exhibit similarity to the adenosine triphosphate (ATP)-binding transport system (ABC transporters), such as *mdl*A and B (Table 2). These proteins contain an

(ATP)-biding cassette (ABC), which is also called a nucleotide-biding domain. They are all integral membrane proteins involved in a variety of transport systems that require ATP. Members of this family include the cystic fibrosis transmembrane conductance regulator, bacterial leukotoxin secretion ATP-binding protein, multidrug resistance proteins, the yeast leptomycin B resistance protein, the mammalian sulfonylurea receptor, and antigen peptide transporter 2 (van Veen et al., 1996).

The genes *ylc*B, *acr*R, *acr*A, and *acr*B (Table 2) exhibit similarity to the MexAB-OprM broadly-specific multidrug efflux system of *Pseudomonas aeruginosa* and *E. coli* (Ma et al., 1996; Poole, 2001; Nikaido and Zgurskaya, 2001). These proteins belong to the RND family and mediate resistance to different drugs, including acriflavine, a macrolide that has homology with flavokinase. In *E. coli*, the *acr*A and *acr*B genes encode a multidrug efflux system that is believed to protect the bacterium against hydrophobic inhibitors. AcrR is a regulator of *acr*AB genes, acting as a modulator to fine tune the level of *acr*AB transcription. This type of gene regulation is uncommon in *E. coli*. The *acr*AB expression is up-regulated under general stress conditions, but this activation is not likely to be directly mediated by the known global stress regulators, such as MarA or SoxS, although elevated levels of these proteins were shown to increase the transcription of *acr*AB (Ma et al., 1996).

CHLORAMPHENICOL

MdfA, the product of the *mdf*A gene homolog identified in the *C. violaceum* genome (Table 2), is an MF-related protein, the over-expression of which results in resistance to a diverse group of cationic and zwitterionic lipophilic compounds, such as ethidium bromide, puromycin, rifampicin, tetracycline, tetraphenylphosphonium, and also to chemically unrelated, clinically important antibiotics, such as chloramphenicol and erythromycin. Some studies have proposed that MdfA is a drug proton antiporter (Edgar and Bibi, 1999).

BICYCLOMYCIN

The bicyclomycin resistance protein (Bcr) is a multidrug transporter from the MF family. The growth-inhibiting drug bicyclomycin is known to be an inhibitor of Rho factor activity in *E. coli* (Yanofsky and Horn, 1995). The *C. violaceum bcr* gene presents similarity to the corresponding gene of *Pseudomonas aeruginosa* (Table 2).

MISCELLANEOUS RESISTANCE GENES

Bacitracin

Bacitracin resistance protein (BacA) is a putative undecaprenol kinase that confers resistance to bacitracin, probably by phosphorylation of undecaprenol (Cain et al., 1993). The *bac*A gene of *C. violaceum* presents similarity to the *bac*A gene of *Magnetospirillum magnetotacticum* (Table 3).

Kasugamycin

The 16S rRNA dimethylase (gene *ksg*A) acts in the biogenesis of ribosomes by cata-

lyzing the dimethylation of two adjacent adenosines in the loop of a conserved hairpin near the 3'-end of the 16S rRNA. Inactivation of *ksg*A leads to resistance to the aminoglycoside antibiotic kasugamycin. The gene found in *C. violaceum* is similar to the *Ralstonia metallidurans ksg*A gene (Table 3) (Vila-Sanjurjo et al., 1999).

Methylenomycin

ORF CV0719 of the *C. violaceum* genome shows homology with the methylenomycin resistance protein (MMR) peptide (Table 3). The predicted mmr-specified protein should be hydrophobic and should present homology at the amino acid level to tetracycline-resistance proteins from both Gram-positive and Gram-negative bacteria. It is suggested that methylenomycin resistance may be conferred by a membrane protein, perhaps controlling the efflux of the antibiotic (Neal and Chater, 1987).

CONCLUDING REMARKS

A remarkable characteristic of *C. violaceum* is the wide range of multidrug resistance and other antibiotic resistance gene homologues in its genome. Secretion of different compounds is a postulated feature of this organism. Certainly, these systems play important roles in the physiology of the bacterium. Interestingly, as these pumps may also transport homoserine lactones, they could have additional roles in cell signaling, such as quorum sensing, a property already demonstrated for *C. violaceum*.

The presence of penicillin-binding proteins in *C. violaceum* is an expected characteristic, because these proteins play an essential role in the cell wall biosynthesis. On the other hand, the presence of beta-lactamase precursors could indicate resistance to beta-lactam antibiotics. This is an important characteristic that probably improves the capacity of *C. violaceum* to compete with other microorganisms in the environment. Additionally, we cannot rule out a function of the beta-lactamases in the rare cases of infections in humans.

The presence of genes that mediate resistance to several other antibiotics was deduced by similarity with counterparts in other bacteria, indicating that drug resistance is an important characteristic of *C. violaceum*.

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