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Microbiology for environmental conservation: a systematic review of bioremediation of heavy metals by *Chromobacterium violaceum*

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Abstract – The release of metals in ecosystems results in not only environmental damage, but also harm to the health and quality of human life. This study aims to compare and discuss the research evolution performed worldwide, which made use of the potential of *Chromobacterium violaceum* for bioremediation of heavy metals. The study consists of a systematic review, limited to research published between 2001 and 2015, using LILACS, PubMed, and SciELO databases. The bioleaching technique was further discussed; gold was the bioremediated substrate most mentioned, and cyanidation was the prevalent phenotypic mechanism. Genetic mechanisms were mentioned in 29.4% of the publications, and among proteins transcribed by *C. violaceum* 18.3% were hypothetical. The adaptive versatility of *C. violaceum* reveals its great biotechnological potential on environmental pollution by heavy metals, favoring environmental conservation. However, the high number of hypothetical ORFs (Open Reading Frame) highlights the need for further research.

Key Words: *Bioremediation; Chromobacterium violaceum; Heavy metals.*

A microbiologia para a conservação ambiental: uma revisão sistemática da biorremediação de metais pesados por *Chromobacterium violaceum*

Resumo – O lançamento de metais nos ecossistemas resulta não somente em danos ambientais, mas também à saúde e qualidade de vida humana. Este trabalho objetiva comparar e discutir a evolução das pesquisas realizadas em âmbito mundial, as quais fizeram uso do potencial biorremediador da *Chromobacterium violaceum* a metais pesados. O estudo consiste em uma revisão sistemática, limitada a trabalhos publicados entre 2001 e 2015, utilizando-se dos bancos de dados LILACS, PubMed e SciELO. A biolixiviação foi a técnica mais discutida; o ouro o substrato biorremediado mais citado e a cianetação o mecanismo fenotípico prevalente. Mecanismos genéticos foram citados em 29,4% das publicações e entre as proteínas transcritas por *C. violaceum* 18,3% eram ditas hipotéticas. A versatilidade adaptativa da *C. violaceum* revela seu grande potencial biotecnológico diante da poluição ambiente por metais pesados, favorecendo a conservação ambiental. Entretanto, o número elevado de ORFs (Open Reading Frame) hipotéticas evidencia a necessidade da realização de novas pesquisas.

Palavras Chave: *Biorremediação, Chromobacterium violaceum; Metais pesados.*

Microbiología para conservación del medio ambiente: una revisión sistemática de la biorremediación de metales pesados por *Chromobacterium violaceum*

Resumen – La liberación de los metales en los ecosistemas no sólo resulta en daños al medio ambiente, sino también la salud y la calidad de la vida humana. Este estudio tiene como objetivo comparar y analizar la evolución de la investigación llevada a cabo en todo el mundo, lo que hizo uso del potencial de biorremediación de Chromobacterium violaceum a metales pesados. El estudio consiste en una revisión sistemática, limitado a las obras publicadas entre 2001 y 2015, utilizando las bases de datos LILACS, PubMed y SciELO. La técnica de biolixiviación se volvió a examinar; el sustrato de oro bioremediated los personajes más citados y la cianuración del mecanismo fenotípica frecuente. Mecanismos genéticos fueron citados por el 29,4% de las publicaciones y entre proteínas transcritas por C. violaceum 18,3% se dijo hipotética. Versatilidad de adaptación de C. violaceum revela su gran potencial de la biotecnología en el entorno de la contaminación por metales pesados y por lo tanto favorece la conservación del medio ambiente. Sin embargo, el elevado número de ORFs (Open Reading Frame) hipotéticas revela la necesidad de nuevas investigaciones.

Palabras clave: *Biorremediación, Chromobacterium violaceum; Metales pesados.*

Introduction

Uncontrolled anthropogenic action from contaminant agents and pollutants wasted in diverse ecosystems has promoted countless environmental impacts on a global scale. Among these contaminants and pollutants, heavy metals have generated great concern, as they consist of persistent elements that are non-degradable, teratogenic, mutagenic, and carcinogenic. Thus, decontamination seems to be a solution of great environmental importance although its use provides enormous quantitative deficits (Weber 2004; Lima, 2008; Sight *et al.*, 2010). This less expressive use of environmental decontamination techniques generates not only impacts on the environment, but also serious consequences to health and people's quality of life, becoming necessary the development, application, and full disclosure of techniques, aimed at reducing the pathogenic potential of ecosystem contamination (Moura, 2001).

The bioremediation term appears in this context as a practical and effective tool, defined as a process in which plants or microorganisms are utilized to remedy or reduce environmental pollutants or degrade toxically harmful substances to the environment. Among the potentially important microorganisms, for the development of this activity, the *Chromobacterium violaceum* is highlighted (Gaylard *et al.*, 2005), which was isolated initially in 1867 (Antônio and Passa, 2004). But only in April of 1880, its discovery was accepted by science, when, accidentally, the Italian researcher Curzio Bergonzini observed the appearance of a violet pigment bacteria and while testing the mechanisms related to putrefaction delay, he suspected *Cromococcus violaceus*. However, after finding the insolubility of this pigment in water, and performing biochemical tests, Bergonzini realized that it was a new organism, called *Cromobacterium violaceum*, and received the addition of the letter 'h' by Zimmerman in 1881, becoming Chromobacterium violaceum (Duran and Meck, 2001). These observations were published in 1882 by Boisbaudran in *Comptes Rendus Hebdomadaires des Seances de L'Academie dês Sciences*, with the following title: "Matiére colorante se formant dans la colle de farine" (Colored material is formed on flour glue) (Antônio and Passa, 2004).

C. violaceum is classified as a Gram-negative, saprophyte, facultative aerobic, rod-shaped Beta-proteobacterium, provided with mobility by a single polar flagellum and lateral or sub-polar flagellums, and present, in solid means, colonies of creamy appearance, and usually with violet color, associated with the pigment violacein synthesis (Dias *et al.*, 2005). Furthermore, it is characterized by a versatile energetic metabolism, where oxidase and reductase enzymes are able to use diverse energy sources, growing in aerobic and anaerobic environments. In aerobic conditions, this bacterium is able to grow in a minimal environment supplemented by simple sugars such as glucose, fructose, galactose, and ribose, using Embden-Meyerhoff and tricarboxylic acid pathway, as well as the glyoxylate cycle. In anaerobic conditions, *C. violaceum* metabolizes glucose with acetic and formic acid production, besides using amino acids and lipids as energy source, thereby giving it a cosmopolitan feature (Antônio and Passa, 2004; Lima-Bittencourt *et al.*, 2007).

This bacterium shows circumtropical distribution, and is generally isolated from terrestrial and aquatic environments, living in tropical and subtropical regions between 35 °S and 35 °N latitudes. The ideal temperature for growth is between 20 ºC and 37 ºC, but some isolated from aquatic environments in the Amazon region may grow at 44 ° C. The high competitiveness and ability of this organism to survive under different environmental stress is related to the production of a variety of secondary metabolites and enzymes, able to detoxify reactive oxygen species, proteins related to tolerance against antimicrobial substances, high temperature, and presence of acids as well as heavy metals (Momen and Hoshino, 2000; Hungria *et al.*, 2005).

Associated with the high spatial distribution and adaptive potential of *C. violaceum*, its high incidence of pathogenicity still stands out. The pathogenic potential of this bacterium was first described by Wooley in 1905, when it was identified as responsible for the death of buffalo by septicemia in the Philippines (Wooley, 1905). Years later, new cases of infections have also been reported in pigs, monkeys, ewes, and dogs (Chattopadhyay *et al.*, 2002). In humans, the first infection case occurred in Malaysia, described by Lesslar in 1927 (Perez *et al.*, 2007). However, *C. violaceum* is normally considered non-pathogenic for humans and may possibly act as an opportunistic pathogen (Durán and Menck, 2001; Crosse *et al.*, 2006).

The infection in humans typically begins with a damaged skin exposure to contaminated soil or water, and has an incubation period from three to fourteen days, affecting mainly young immunocompromised individuals. The clinical features begin with cellulitis, lymphadenitis, skin wounds and visceral abscesses, most often hepatic, kidney, and pulmonary; and urinary tract infection, gastroenteritis, osteomyelitis, sinusitis, and meningitis may still occur (Sirinavin *et al.*, 2005; Teoh *et al.*, 2006; Martinez and Mattar, 2007; Manjunath, 2007) . The symptomatology usually progresses to severe sepsis and death in 24 to 72 h. The quick evolution and ineffective treatment with antibiotics result in a fatality rate of over 60% (De Siqueira *et al.*, 2005). The infection diagnosis is made by culturing blood or abscess fluid, and biochemical identification (Chang *et al.*, 2007), although there are studies for the development of diagnostic techniques per PCR (Polymerase Chain Reaction) (Scholz *et al.*, 2006).

Despite pathogenic implications attributed to this bacterium, several studies highlight *C. violaceum* as a microorganism with diverse industrial, pharmaceutical, and ecological applications. Many researchers have reported the presence of genes involved in resistance to arsenic, cyanide, and iron, as well as capable of reducing halogen compounds to less toxic forms (Carepo *et al.*, 2004; Azevedo *et al.*, 2008; Lima *et al.,* 2014). Thus, facing several biotechnological applications assigned to this microorganism, in 2001, the nucleotide sequencing national grid, inserted in the Brazilian genome project, began sequencing the ATCC 12472 lineage of *C. violaceum,* isolated from aquatic environments located in Mentekeb and Malaysia. This sequencing was finalized in 2003, thereby enabling the development and application of efficient techniques to combat and mitigate pollution of ecosystems (Vasconcelos *et al.*, 2003).

Therefore, a better understanding of the dynamics of *C. violaceum* bioremediation of heavy metals is required around the world and in diverse environments, aiming, with this knowledge, to allow improvement and application directed toward prevention and environmental pollution control. Thus, this study was structured from a systematic review of specialized references on the use of *C. violaceum* in bioremediation techniques for heavy metals, utilized worldwide in diverse environments, aiming to analyze, compare, and describe the temporal evolution of developed research on this theme, from the sequencing of *C. violaceum* that began in 2001 to the present day.

Materials and methods

This study consists of a systematic review, limited to research published between 2001 to 2015, whose theme approached the use of *Chromobacterium violaceum* in bioremediation of heavy metals in diverse environments and published anywhere in the world. The article search was performed in LILACS, PubMed, and SciELO databases. A systematized theoretical review was conducted, where keywords in Portuguese, English, and Spanish, proposed by DECs (Descritores de Assunto em Ciências da Saúde - Subject Keywords in Health Sciences), belonging to the BVS database (Biblioteca Virtual em Saúde - Virtual Health Library), were divided into three key terms: 1) *Chromobacterium violaceum*; 2) bioremediation and its synonyms; and 3) metals and related terms, as shown in Table 1.

Table 1 - Keywords utilized in the theoretical survey.

	Essential Keywords	
Portuguese	**English**	**Spanish**
	TERM 1	
	Chromobacterium violaceum	
	TERM 2	
Bioremediação	***Bioremediation***	***Bioremediación***
Sinônimos:	**Synonims:**	**Sinónimos:**
Remediação;	Remediation;	Remediación;
Metabolização;	Metabolism;	Metabolismo;
Mineralização;	Mineralization	Mineralización
Transformação;	Transformation;	Transformación;
Redução;	Reduction;	Reducción;
Remoção;	Removal;	Remoción;
Eliminação;	Elimination;	Eliminación;
Degradação microbiana;	Microbial Degradation;	Deterioro Microbiano;
Degradação biológica;	Biological Degradation;	Degradación Biológica;
Biorreparação e biolixiviação	Biorestauration and bioleaching	Biorreparación y biolixiviación
	TERM 3	
Metal pesado	***Heavy Metal***	***Metale Pesado***
Sinônimos:	**Synonims:**	**Sinónimos:**
Metal;	Metals;	Metales;
Resíduo metálico	Metallic Waste	Residuo Metálico

Excluded from this review were studies not complying with the inclusion criteria, those that had only cited the above-mentioned keywords but whose focus of research was not bioremediation related to *C. violaceum* and heavy metals, those whose bioremediator organism was not *C. violaceum*, and/or those in which the degraded substrate differed from heavy metals.

Finally, we tried to compare and discuss the temporal evolution research performed worldwide, which made use of the potential of *C. violaceum* for bioremediation of heavy metals in the last 14 years. Highlighted in our review were the countries with the highest prevalence, the main isolation environments of this microorganism, the main bioremediation techniques addressed, the most tested heavy metals, the main phenotypic and genotypic resistance mechanisms of *C. violaceum* on these metals, as well as the main genes associated with them.

Results and discussion

From the theoretical review performed in all databases, 1053 studies were obtained. After application of the exclusion criteria, we found 17 publications, consisting of 16 articles (94.1%) and 1 master's thesis (11.8%). As for the publications list with database evaluated, 11 (64.8%) were from the PubMed and 3 (17.6%) from LILACS and SciELO, each. The studies were published between 2001 and 2013 in the North America and South America Americas(41.2%), Europe (17.6%), Asia (35.3%), and Oceania (5.9%). Brazil had the largest number of studies associated with the theme (35.3%) (Table 2).

Table 2 - Searchable databases, type and year of publication, prevalence of continents and countries inserted in the studied subject.

Features	N	%
Database		
PubMEd	11	64.8
LILACs	3	17.6
Scielo	3	17.6
Type of Publication		
Article	16	94.1
Other (monographs, dissertations and thesis)	1	5.9
Year of Publication		
2001, 2007, 2008, 2009, 2011	1 each	23.5
2006, and 2012	2 each	35.4
2013	3 each	17.6
2004	5 each	23.5
Continents		
America	7	41.2
Europa	3	17.6
Asia	6	35.3
Oceania	1	5.9
Countries		
Brazil	6	35.3
Switzerland	3	17.6

North Korea	3	17.6
United States	1	5.9
Japan	1	5.9
Singapore	1	5.9
India	1	5.9
Australia	1	5.9
Total	17	100

Assessing the methodology addressed in each selected study, we observed that 76.4% utilized the standard strains *C. violaceum* associated with the adaptation and bioremediation mechanisms of heavy metals. The studies which utilized standard strains frequently mentioned ATCC 12472 (46.1%), followed by DSMZ 30191 (30.8%), and NBRC 12614, MTCC 2656 and KTCT strains, mentioned in 7.7% of the research. Only 23.6% of the studies did not specify the type of strain, and one publication made use of isolated wild strains from an aquatic environment, in well water.

C. violaceum adaptation to heavy metals was discussed in all selected studies, and among the bioremedied substrates, gold was the most researched (35.6%), followed by copper (13%); iron (9.8%); zinc, silver, arsenic, and nickel, 6.4% each; while platinum, aluminum, manganese, cadmium and mercury were less prevalent, each mentioned in 3.2% of publications.

Phenotypic adaptation was the most commonly addressed mechanism (70.6%), and only cyanide production was highlighted in all the studies by bioleaching technique. Only five studies (29.4%) mentioned and/or described genetic mechanisms associated with the adaptation of *C. Violaceum* to iron, arsenic, magnesium, zinc, cadmium and mercury heavy metals, as well as the correlation between bioleaching processes associated with hydrogen cyanide synthesis by this microorganism. As for the genetic mechanisms, 93 ORFs were mentioned associated with adaptive capacity and *C. violaceum* bioremediation of heavy metals. Among these, 88.8% made reference to adaptation and metabolization of iron, 4.3% to arsenic and zinc each, and 1.1% to cadmium, mercury and manganese metals, each, according to Tables 3, 4 and 5.

Table 3 - Proteins associated with metal metabolism in *C. violaceum*.

Gene	ORF	Definition	Metal
arsR	2438	Transcription regulator protein, arsenical resistance operon repressor	Arsenic
arsC	2439	arsenate reductase	Arsenic
arsB	2440	arsenic resistance membrane protein ArsB/ACR3	Arsenic
hcnC	1682	hydrogen cyanide synthase HcnC	*
hcnB	1683	hydrogen cyanide synthase HcnB	*
hcnA	1684	hydrogen cyanide synthase HcnA	*
fur	1797	ferric uptake regulation protein	Iron
fepA	2230	enterobactin-iron outermembrane receptor protein	Iron

fepC	2234	enterochelin ABC transporter ATP-binding protein	Iron
fepD	2236	iron-enterobactin transporter membrane protein	Iron
fepG	2235	iron-enterobactin transporter permease	Iron
fhuA	2251	ferrichrome-iron outermembrane receptor protein	Iron
fhuC	1487 1560 1793	iron ABC transporter ATP-binding protein	Iron
znuA	3064	high-affinity zinc transport system substrate-binding protein	Zinc
znuB	3065	high-affinity zinc transport system permease	Zinc
znuC	3066	zinc transport system ATP-binding protein	Zinc
zntA	1154 3478	lead,cadmium,zinc and mercury transporting ATPase	Cadmium, Zinc and Mercury
mntH	0576	manganese transport protein MntH	Manganese

* *gene associated with regulation and hydrogen cyanide synthesis by C. violaceum, playing an important role in bioleaching processes of heavy metals.*

From the protein function point of view, it was observed that most of the described proteins (66.7%) had adaptive potential of resistance to heavy metals, performing functions of regulation and/or metabolization of these elements, while 18.3% of proteins were considered hypothetical and whose sequencing has not been performed or completed, and 11.8% had the carrier function of heavy metals. In addition, it was observed in the literature that *C. violaceum*, when exposed to iron concentrations, tends to express several proteins with transport functions, regulation, and metabolization of these elements, which are subdivided into non-hypothetical (Table 4) and hypothetical (Table 5).

Table 4 - Non-hypothetical protein expressed in *C. violaceum* cultivated in iron presence.

Gene	ORF	Definition
-	0148	transcriptional regulator
-	0124	inhibitor of septum formation
glpK	0251	glycerol kinase
aldB	0393	aldehyde dehydrogenase
Soj	0475	chromosome partitioning protein ParA
Atpf	0668	ATP synthase F0 subunit B
Glms	0677	glucosamine--fructose-6-phosphate aminotransferase
-	0889	methyl-accepting chemotaxis protein
pstS	0938	phosphate ABC transporter substrate-binding protein
nuoD	0944	NADH dehydrogenase subunit D
ahcY	0965	S-adenosyl-L-homocysteine hydrolase

-	1029	GntR family transcriptional regulator
-	1080	methyl-accepting chemotaxis protein
gst1	1164	glutathione S-transferase family protein
-	1222	aldehyde dehydrogenase
ctaQ	1292	carboxypeptidase Taq
phoA2	1513	alkaline phosphatase
-	1541	3-oxoacyl-ACP synthase
-	1646	porin
cysS	1746	cysteinyl-tRNA synthetase
metY	1934	O-acetylhomoserine (thiol)-lyase
caiA	2084	acyl-CoA dehydrogenase
-	2097	nitrilase
trpE	2179	anthranilate synthase component I
-	2181	short chain dehydrogenase
Rpe	2182	ribulose-phosphate 3-epimerase
-	2481	amino acid ABC transporter
-	2802	peptide synthetase
pepA	2914	leucyl aminopeptidase
-	2932	signal peptide protein
flaD	3011	flagellin D
-	3012	serine carboxypeptidase
Ugd	3041	UDP-glucose dehydrogenase
nagD	3244	N-acetylglucosamine metabolism protein
vioA	3274	vioA - tryptophan 2-monooxygenase
acsA	3282	acetyl-CoA synthetase
aceB	3304	malate synthase
Pgm	3352	phosphoglyceromutase
Prc	3354	carboxy-terminal processing protease
cysI	3573	sulfite reductase hemoprotein subunit beta
-	3605	hydrolase
purM	3615	phosphoribosylaminoimidazole synthetase
rpsF	3640	30S ribosomal protein S6
rpoS	3682	RNA polymerase sigma factor RpoS
trpS2	3715	tryptophanyl-tRNA synthetase
greA	3801	transcription elongation factor
-	3814	type II secretion system protein
goaG	3926	4-aminobutyrate aminotransferase
-	3943	ABC transporter ATP-binding protein
hoxX	4142	hoxX-like protein

ftsE	4204	cell division ATP-binding protein ftsE, ABC transporter ATP-binding protein
-	4239	amidase
-	4276	glutamate-cysteine ligase
-	4392	ABC transporter

Table 5 - Hypothetical protein expressed in *C. violaceum* cultivated in iron presence.

Hypothetical protein expressed in *C. violaceum* cultivated in iron presence.		
Gene	**ORF**	**Definition**
-	0008	hypothetical protein
-	0090	hypothetical protein
CV_0856	0856	hypothetical protein
CV_0884	0884	hypothetical protein
CV_1082	1082	hypothetical protein
CV_1175	1175	hypothetical protein
CV_1543	1543	hypothetical protein
CV_1628	1628	hypothetical protein
CV_1790	1790	hypothetical protein
CV_2374	2374	hypothetical protein
CV_2376	2376	hypothetical protein
CV_3001	3001	hypothetical protein
CV_3099	3099	hypothetical protein
CV_3694	3694	hypothetical protein
CV_3824	3824	hypothetical protein
CV_3850	3850	hypothetical protein
CV_4107	4107	hypothetical protein

Among the main bioremediation techniques discussed, bioleaching was the most frequently addressed, mentioned in 70.6% of the studies published, while other studies did not specify the technique. The applicability of bioremediator techniques was directed to several environments and materials. Among these, the compounds from electronic residues were most frequently related to remediation of heavy metals by *C. violaceum* (35.3%) evaluated separately and/or together with other compounds. Similarly, compounds originating from automotive catalytic converters were described in 11.8% of the research, while those from printed circuit boards were mentioned in only 5.9%.

Among the selected publications, 17.6% mentioned the industrial sector, especially the mining industry, as the probable environment for applicability of bioremediator techniques, while other studies only described the techniques without highlighting the environment for application. In the obtained review, 23.5% of the studies were published in 2004, one year after the sequencing of *C. violaceum*, finalized in 2003. In addition, the Brazil pioneerism in research with this microorganism, associated with favorable weather conditions for adaptation and isolation, may explain the higher prevalence of studies associated with the theme (35.3%).

The isolation of this microorganism has been reported worldwide. Countries such as Hong Kong, India, Australia, Colombia, the United States, Nigeria and South Africa, located in tropical and subtropical regions show favorable conditions to the growth of this organism (Currie and Carapetis, 2000; Martinez *et al.*, 2000; Chattopadhyay *et al.*, 2002; Ray *et al.*, 2004; Dias *et al.*, 2005; Teoh *et al.*, 2006; Pérez *et al.*, 2007; Bosch *et al.*, 2008). This also explains the performance of studies associated with this issue in countries such as North Korea (17.6%), as well as Japan, India, Singapore, Australia, and the United States (5.9% each).

In Brazil, *C. violaceum* is present in three main ecosystems: the Amazon Forest, Cerrado, and Atlantic Forest (Byamukama *et al.*, 2005; Lima-bittencourt *et al.*, 2007; Dall'agnol *et al.*, 2008). There are also reports of *C. violaceum* isolation in other locations in Brazil, such as Rio Negro (Black River) and sand banks located in Amazon region, as well as in rivers and soils of Serra do Cipó National Park (MG), thus showing the high adaptability of this bacterium (Dias *et al.*, 2002).

Furthermore, studies have highlighted the high adaptability of *C. violaceum* strains isolated from aquatic environments to aluminium, copper, manganese and zinc heavy metals (Sumita *et al.*, 2007). Another study, in turn, identified 35 genes associated with transport and storage of iron in this organism, as well as several other genes related to the complexion and decontamination of heavy metals (Vasconcelos *et al.*, 2003). This highlights the importance of the development and application of combat and mitigation biotechnology techniques for environmental problems, resulting from the release of metallic waste, such as, bioremediation.

Diverse studies show the use of *C. violaceum* for bioremediation of heavy metals, emphasizing its bio-decontamination potential, and/or as bio-indicator. A total of 496 Open Reading Frames (ORFs) were identified in *C. violaceum, for instance,* membrane proteins related to transport, among which are highlighted several transport proteins of heavy metals, thus indicating the versatility of this organism in controlling the concentration of diverse substances by intracellular means (Durán and Menck, 2001; Vasconcelos *et al.*, 2003; Grangeiro *et al.*, 2004).

The genetic sequencing of *C. violaceum* disclosed the presence of a genome consisting of a circular chromosome with 4,751,080 base pairs, with 89% of the coding regions. Of these ORFs, approximately 61% encode known proteins. The other 39% are hypothetical proteins, which are divided into conserved proteins and exclusive of this bacterium. The genome shows 98 tRNA genes, which are capable of transcribing carriers for 20 amino acids of the cell. rRNA genes are grouped into eight operons, and they all contain identical sequences of the coding region, according to the *Brazilian National Genome Project* BNGP 2003 (Vasconcelos *et al.*, 2003).

In addition, 539 ORFs were identified in the *C. violaceum* genome, which encode proteins involved in metabolite transport. Of these, 489 ORFs encode protein sequences showing significant similarity to sequences of membrane proteins, which are related to transport in other organisms. The other 50 ORFs were characterized as hypothetical and/or conserved hypothetical. This significant number of potential carriers may be related to the fact that *C. violaceum* is found in different environments of tropical and subtropical regions, requiring adaptation to different external conditions. What still stands out is its metabolic capacity to express proteins when facing stress situations, related to the presence of heavy metals (Grangeiro *et al.*, 2004).

Table 6 - *C. violaceum* genome characteristics.

C. violaceum Genome	
Length (pb)	*4,751,080*
Content (Guanine + Cytosine)	64.83%
ORFs	4,431
Coding regions (%)	89%
Average length per ORF (pb)	954
Known proteins	2,717
Conserved hypothetical proteins	958
Exclusive hypothetical proteins	756
Operons of rRNAs	8 x (16S-23S-5S)
Genes of tRNAs	98

Metabolic interactions among heavy metals and microorganisms affect the chemical composition of those elements, changing characteristics such as solubility, mobility, bioavailability and toxicity; therefore, the use of *C. violaceum* may contribute decisively for the implementation of recovery, mobilization, immobilization, or metallic pollutant detoxification techniques. In this context, the use of *C. violaceum* appears to be an important tool for treating contaminated areas with heavy metals, since such bacteria are involved in several biogeochemical cycles and many of them show adaptive, plasmidial, and chromosomal mechanisms in elevated concentrations of metals, as well as conversion of toxic forms of these elements into less toxic forms (Mukhopadhyay *et al.*, 2002).

Regarding such high adaptive capacity of *C. violaceum* to heavy metals, diverse bioremediator techniques have been described in the literature, among which bioleaching is the most commonly mentioned. The bacterial leaching or bioleaching may be defined as a biotechnological process based on the use of microorganisms able to solubilize metal through oxidation of metallic sulfides (Pradhan *et al.*, 2008; Pradhan and Kumar, 2012; Shin *et al.*, 2013). In this process, the bacterial adaptation mechanisms become indispensable to the complexion and bioremediation of the heavy metals treated (Bevilaqua *et al.*, 2002; Haghshenas *et al.*, 2009).

Studies suggest the possible existence of two adaptive mechanisms of bacterial response during bioleaching technique application. The first mechanism is called "direct mechanism," and is based on microbial adhesion to the metal surface, followed by mineral dissolution through enzymatic reactions. The second mechanism is based on electrostatic interaction of extracellular polysaccharide (EPS), synthesized by the bacteria to the treated mineral, through oxidative reactions (Sand *et al.*, 2001). *C. violaceum* shows great applicability in bioleaching techniques, and has an Operon hcnABC associated with the production of Hydrogen Cyanide (HCN), which interacts with diverse metals, complexing them to stable and water-soluble forms. It is, therefore, of great value in gold solubilization processes, e.g., avoiding environmental pollution by the traditional process, which uses mercury to recover this precious metal, thus constituting an important bioremediation strategy in areas contaminated by gold-digging (Campbell *et al.*, 2001; Durán and Menck, 2001; Carepo *et al.*, 2004; Duarte *et al.*, 2004; Faramarzi *et al.*, 2004; Brandl and Faramarzi, 2006; Kita *et al.*, 2006; Brandl *et al.*, 2008; Fairbrother *et al.*, 2009).

Studies have shown that *C. violaceum* was able to solubilize, for seven days, 15% of gold and copper of printed circuit board pieces (Faramarzi *et al.*, 2004). The printed circuit boards may be found in most all-electronic equipment, being composed generally of ceramic or metallic polymers. Moreover, the growing waste disposal of electronic equipment has provided a bioaccumulation of these elements, causing large environmental problems, mainly due to the release of metallic waste from the industry (Veit et al., 2006; Guo and Xu, 2009; Chi *et al.*, 2011).

The development of the industrial sector is a major contributor to the pollution problem of the ecosystems, whether by negligence in waste treating or by frequent accidents and carelessness. Besides, environmental pollution due to the presence of metals has become recurrent in modern society, and heavy metals such as iron, copper, cadmium, zinc, lead, nickel and chromium are often found in the environment, presenting critical toxicity, especially to aquatic and terrestrial life, including humans. The review performed in this study demonstrated that these elements have been reported, with prevalence of 13% for copper and 9.8% for iron. Gold was mentioned in 35.6% of the studies. Although it is not commonly released into the environment, gold has a high economic value, justifying the large number of publications directed to this element (Tay *et al.*, 2013).

Heavy metals are among the main agents responsible for environmental pollution, being involved in cytotoxicity, genotoxicity, and carcinogenesis. The environmental pollution by these elements makes possible the acidification in aquatic environments, accelerating the heavy metal solubilization process and making these environments selective to diverse microorganisms (Desoize, 2003; Waisberg *et al.*, 2003).

In this way, the development and application of bioremediator techniques are required, as well as the use of microorganisms that present high adaptive capacity to heavy metals. Highlighted among these is *C. violaceum*, which can possibly mitigate and/or combat the negative environmental impacts caused by such elements.

Conclusion

The literature review showed phenotypic and genotypic features of *C. violaceum* associated with the resistance and bioremediator capacity to heavy metals, and emphasizes the great importance of its biotechnological potential, attributed to the use of this bacterium in the control and mitigation processes of the problematic environmental pollution caused by heavy metals. In addition, the adaptive versatility of this microorganism allows a circumtropical distribution, evidenced by several studies performed in the American, European, Asian and Oceania continents, and, consequently, makes possible the application of bioremediator techniques through the use of *C. violaceum* in diverse environments. However, despite the biotechnological importance of *C. violaceum*, the systematic review found an even higher number of hypothetical ORFs related to the adaptation of this organism to heavy metals, and highlights the need for further research aimed at a better understanding of the *C. violaceum* dynamics on these elements.

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