EXPRESSION OF SYNTHETIC PHYTOCHELATIN EC20 IN *E. COLI* INCREASES ITS BIOSORPTION CAPACITY AND CADMIUM RESISTANCE

EXPRESSÃO DA FITOQUELATINA SINTÉTICA EC20 EM E. COLI AUMENTA SUA CAPACIDADE DE BIOSSORÇÃO E RESISTÊNCIA AO CÁDMIO.

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ABSTRACT: In this study *E. coli* recombinant clones that express the EC20 synthetic phytochelatin intracellularly were constructed. The increasement of Cd^{2+} biosorption capacity, and, also, the increasement of resistance to this toxic metal were analyzed. A gene that encodes the synthetic phytochelatin EC20 was synthesized *in vitro*. The *EC20* synthetic gene was amplified by PCR, inserted into the DNA cloning vectors pBluescript®KS^+ and pGEM®-TEasy, and also into the expression vectors pTE [pET-28(a)® derivative] and pGEX-T4-2®. The obtained recombinant plasmids were employed for genetic transformation of *E. coli*: pBsKS-EC20 and pGEM-EC20, they were introduced into DH10B and DH5α strains, similarly to pTE-EC20 and pGEX-EC20 that were introduced into BL21 strain. The EC20 expression was confirmed by SDS-PAGE analysis. The recombinant clones' resistances to Cd^{2+} were determined by MIC analyses. The MIC for Cd^{2+} of DH10B/pBKS-EC20 and DH10B/pGEM-EC20 were 2.5 mM (EC20 induced), and 0.312 mM (EC20 repressed); respectively, 16 and 2 times higher than the control DH10B/pBsKS (0.156 mM). The MIC for Cd^{2+} of BL21/pTE-EC20 was 10.0 mM (EC20 induced) and 2.5 mM (EC20 repressed), compared with the control BL21/pTE which was only 1.25 mM. Analysis of ICP-AES showed that BL21/pGEX-EC20, after growth on the condition of EC20 expression, absorbed 37.5% of Cd^{2+}, and even when cultured into the non-induction condition of EC20 expression, it absorbed 11.5%. These results allow the conclusion that recombinant *E. coli* clones expressing the synthetic phytochelatin EC20 show increased capacity for Cd^{2+} biosorption and enhanced resistance to this toxic ion.

KEYWORDS: *Escherichia coli*. Phytochelatin. Biosorption. Bioremediation. Cadmium.

INTRODUCTION

Recently, industrial and other human activities have been generating environmental pollution in a never observed amounts, creating demands for the development of new remediation techniques. Polluting organic materials can, in most cases, be completely degraded, and that is called bioremediation (GAYLARDE; BELLINASO; MANFIO, 2005; PERELO, 2010). However, metal pollutants tend to persist indefinitely in the environment thus threatening ecosystems as they accumulate along the food chain (AKPOR; MUCHIE, 2010).

The speciation of a metal (different chemical forms/species metal can exist in nature) determines its bioavailability, mobility and destination (REEDER; SCHOONEN; LANZIROTTI, 2006; LADEIRA et al., 2014).

Conventional chemical or physical wastewater treatment are often inappropriate to reduce metal concentrations to the acceptable regulatory standards, and in general, they are cost-expensive and result on hazardous products (AKPOR; MUCHIE, 2010; GIRIPUNJE; FULKE; MESHRAM, 2015).

A promising alternative that grows on demand and development is the use of biomaterials for biosorption of toxic heavy metals. These biomaterials are named as biosorbents (VOLESKY; HOLAN, 1995; GAVRILESCU, 2004; GADD, 2009; WANG; CHEN, 2009; GUPTA; NAYAK; AGARWAL, 2015), they are beginning to be used in bioremediation of metal contaminated waters (GAVRILESCU, 2004; AKPOR; MUCHIE, 2010; AYANGBENRO; BABALOLA, 2017). Compared to traditional physicochemical techniques, bioremediation of toxic metals presents advantages such as lower costs, superior performance and safety, besides being environmentally friendly (GADD, 2009; WANG; CHEN, 2009; GUPTA; NAYAK; AGARWAL, 2015; AYANGBENRO; BABALOLA, 2017).

There are a wide variety of microorganisms (bacteria, fungi, yeasts and algae) that have good potential for use in bioremediation processes as

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biosorbents for heavy metals (VOLESKY; HOLAN, 1995; WANG; CHEN, 2009; AYANGBENRO; BABALOLA, 2017).

As most heavy metals are cationic, this determinate their sorption into negatively charged functional hydroxides (-OH) or thiol (-SH) groups present on the surfaces of the biosorbents. In the cells, several groups interact with metal species allowing its capture, including cysteine SH group (GADD, 2009; WANG; CHEN, 2009). All living cells, in the presence of toxic heavy metals, produce cysteine-rich peptides such as glutathione (GSH), phytochelatins (PCs), and metallothioneins (MTs) that bind metal ions (such as Cd^{2+}, Cu^{2+}, Cr^{3+}, Cr^{5+}, Hg^{2+}, Mn^{2}, Pb^{2+}), turning them into biologically inactive forms (STILLMAN, 1995; COBBETT; GOLDSBROUGH, 2002).

The best-efficient heavy metal-binding molecules are the phytochelatins (PCs) which are repetitions of the γ-GluCys dipeptide followed by a terminal Gly [$(\gamma\text{-GluCys})_n$-Gly; n=2-11)]. So, PCs are oligomers of glutathione enzymatically linked by gama-type ligation (MEHRA; MULCHANDANI, 1995; COBBETT; GOLDSBROUGH, 2002).

Considering the advantage PCs offer as they are short cysteine-rich peptides, Bae et al. (2000) constructed recombinant *E. coli* strains expressing synthetic phytochelatins (ECs; αGlu-Cys)nGly, n=8-20) by the normal bacterial transcription ribosomal machinery. These ECs were expressed in fusion with the outer membrane protein A (OmpA) and became linked onto the bacterial cell surface. The resulting recombinants accumulate a substantially higher amount of Cd^{2+} than the wild-type cells (BAE et al., 2000). After that, genetic engineered bacteria expressing phytochelatin biosynthesis genes (SAUGE-MERLE et al., 2003; WAWRZYŃSKA et al., 2005) or synthetic phytochelatin genes are emerging as new tools for environmental remediation of heavy metals (BAE; MEHRA; MULCHANDANI; 2001; BIONDO et al., 2012; CHATURVEDI; ARCHANA, 2014; YANG et al., 2017).

On this study, the construction of recombinant *Escherichia coli* strains expressing the synthetic phytochelatin EC20 intracellularly was described, as well as the consequent increases on the capacity of Cd^{2+} biosorption and the resistance to this toxic ion of these recombinant clones in comparison to the original phenotypes of non-transformed strains.

MATERIAL AND METHODS

Bacterial strains and growth conditions

In this study we used the *E. coli* strains DH5α, DH10B (SAMBROOK; RUSSELL, 2001) and BL21-DE3 (Novagen®). The cell growth was carried out at 37 °C in liquid Luria Bertani medium - LB and in low-phosphate medium - MJS (UEKI et al, 2003). When required, the mediums were supplemented with 60 μg/mL carbenicillin or 50 μg/mL kanamycin (SAMBROOK; RUSSELL, 2001). All mediums were elaborated with components and microbiological grade salts supplied, respectively, by Difco® and Sigma-Aldrich/Merck®. Cultures in solid and liquid media were incubated at 37 °C in incubator chamber and in shaker (180 rpm), respectively.

In vitro construction of the synthetic phytochelatin *EC20* codifying gene

The *EC20* synthetic gene was constructed employing the strategy described by Bae et al. (2000) and standard molecular protocols (SAMBROOK; RUSSELL, 2001). Some GAA and TGT codons were changed for GAG and TGC, to prevent unwanted hybridization. The oligonucleotides EC-A: 5'TTT*GGATCC*ATGGAATGTGAATGTGAATGT GAATGTGAATGTGAATGTGAATGTGAGTGT GAATGT**GAGTGCGAATGCGAA**3' (site *Bam*HI-italicized), and EC-B: 5'TTT*AAGCTT*TTAACCACATTCACATTCACAT TCACATTCACATTCACATTCGCATTCACATT CGCA**TTCGCATTCGCACTC**3' (site *Hind*III-italicized) were mixed, boiled, and cooled for hybridization of the bold sequences. The mixture was treated with the Klenow fragment of DNA polymerase enzyme. This double strand was used as template in a PCR reaction with the primers ec-a (5'-tttggatcca-3') and ec-b (5'-tttaagcttt-3'), and the enzyme taq-DNA polymerase, resulting in the DNA fragment *Bam*HI→*Hind* III (*EC20* synthetic gene, 141bp) (Figure 1-a). All nucleotides and enzymes were purchased from Promega® and Fermentas®, respectively. The thermocycler machine used was "MJ Research-model PTC-200".

Cloning the *EC20* synthetic gene

The *EC20* synthetic gene and the plasmid pGEM-TEasy (Promega®) were mixed with T4-DNA ligase, and that ligation mixture (SAMBROOK; RUSSELL, 2001) was used on the genetic transformation of *E. coli* DH10B strain. Plasmids isolated from some randomly selected recombinant clones were digested with *Bam*HI and *Eco*RI (site present in the pGEM-TEasy plasmid) and analyzed in a gel submitted to electrophoresis,

to select the plasmid pGEM-EC20 (Figure 1-b). For the subsequent plasmids' constructions, the *EC20* DNA fragment was obtained by PCR using as template PGEM-EC20, primers T3 and T7 (Promega®), as well as the enzyme High Fidelity DNA polymerase**.** The amplicon was digested with *Bam*HI-*Hind*III (Figure 1-c) and linked to the cloning plasmid pBsKS [pBluescriptKS(+)] (Stratagene®) and to the expression vector pTE [a pET-28(a) - Novagen® derivative without the His-tag codifying region: the original plasmid was digested with *Nco*I and *BamH*I, treated with Klenow DNA polymerase, and relinked using DNA T4 ligase], both pre-digested with the same enzymes, using DNA T4 ligase. The PCR *EC20* amplicon flanked by *Bam*HI and *Eco*RI (Figure 1-c) was also linked to vector pGEX-T4-2 (Promega®) previously digested with the same enzymes, using DNA T4 ligase (Figure 1-d).

The plasmids pGEM-EC20 and pBsKS-EC20 were used on the genetic transformations of *E. coli* DH5α and DH10B strains, respectively. The expression recombinant plasmids pTE-EC20 and pGEX-EC20 were used for genetic transformations of *E. coli* BL21-DE3 (Merck®) strain. This resulted into recombinant clones DH5α/pGEM-EC20, DH10/pBsKS-EC20, BL21/pTE-EC20 and BL21/pGEX-EC20.

DNA sequencing

For DNA sequencing, we used T3 and T7 (Promega®) primers, BigDye® sequencing-Kit, sequencing machine ¨ABI 3730 DNA Analyzer¨, and the software ¨Sequencing Analysis 5.3.1 with the Base Caller KB¨ from Applied Biosystems®.

Protein expression methods

The recombinant clones BL21/pTE-EC20 and BL21/pGEX-EC20 were cultured in LB medium+IPTG (final concentration 800 µM), at 37 ºC, in shaker (180 rpm), until Abs_{600nm} 1.0. The total amount of protein was extracted and analyzed by SDS-PAGE. Since EC20 protein has only 4.6 kDa, 17.5% acrylamide was used (LAEMMLI, 1970; SAMBROOK; RUSSELL, 2001).

Heavy Metals Resistance Determination

Analytical-grade $CdCl_2.2.5H_2O$ (Merck®) was used to prepare 0.1 M stock solution and was sterilized by membrane filtration (0.22 µm, Millipore®). Deionized water was used throughout the study. Recombinant clones were pre-cultured in 3.0 mL of LB medium plus antibiotic, incubated at 200 rpm, at 37 ºC, for 24 hours. For *EC20* expression induction it was added 2 mM IPTG (final concentration 800 µM), and for its repression it was added 2% glucose (SAMBROOK; RUSSELL, 2001). From those pre-cultures, 25 µL was inoculated into 25 mL of fresh liquid MJS medium with the same supplement additions. Each one of those cultures were distributed in 10 tubes: 4.0 mL were poured into the first tube and 2.0 mL in the remaining tubes. To the first tube it was added $CdCl_2$ to a final concentration of 10 mM, and from that, 2.0 mL were transferred to the next tube, successively. The tubes were incubated at 37 °C, 200 rpm, for 24 hours. The minimal inhibitory concentration (MIC) of Cd^{2+} for the clones was determined by visual observation of the turbidity (ANDREWS, 2001). All experiments were performed in duplicates.

Heavy Metals Bioaccumulation

In duplicates, recombinant clones were cultured in 5.0 mL of LB medium plus antibiotic and incubated at 37 °C, 200 rpm, for 16 hours. 30 µL were inoculated in 30 mL of fresh medium with the same composition. The initial cell concentration was standardized at Abs_{600nm} 0.15. After 1-hour incubation, IPTG (final concentration 800 µM) was added for the EC20 expression induction. The cultures were incubated until Abs_{600nm} 0.5. The cells were harvested by centrifugation (4 ºC, 6000 g, 20 min). The pellet cells were suspended in 50 mL $CdCl_2$ 1.000 µM, incubated at 37 °C, 200 rpm, for 2 hours, and centrifuged (4 ºC, 6.000 g, 20 min). The remaining Cd^{2+} in the supernatant was determined by Inductively Coupled Plasma Atomic Emission Spectrometry - ICP-AES (ESPECTRO®-ARCOS), the calibration curve was made with a 1000 ppm cadmium mono-element standard solution.

RESULTS

The DNA fragment codifying EC20 synthetic phytochelatin was constructed *in vitro* and amplified by PCR (Figure 1-a). The *EC20* synthetic gene was inserted into two cloning plasmids pGEM-TEasy (Promega®) and pBluescriptKS(+) (Stratagene®) resulting in the recombinant plasmids pGEM-EC20 (Figure 1-b) and pBsKS-EC20 (Figure 1-c). These recombinant plasmids were used in the genetic transformation of *E. coli* DH10B and DH5α strains. *EC20* (Figure 1-c) was also inserted into the expression vectors pTE (a pET-28(a)-Novagen® derivative constructed in this research) and pGEX-T4-2-(Promega®) (Figure 1-d) resulting in the recombinant plasmids pTE-EC20 and pGEX-EC20.

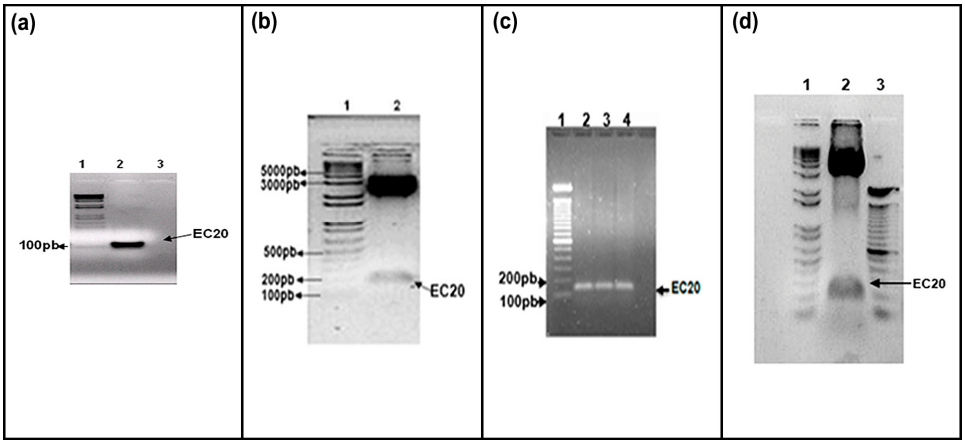

Figure 1. Migration profiles of DNA fragments on 1.0% agarose gels after electrophoresis and subsequent staining with 0.02% ethidium bromide: **(a)** 1-Molecular marker 100 pb (Invitrogen®), 0.8 µg; 2-DNA fragment synthetic phytochelatin EC20 after PCR amplification. **(b)** 1- Molecular marker 1 kb (Invitrogen®), 0.8 µg; 2-PGEM-EC20 digested with *Bam*HI and *Eco*RI. **(c)** 1- Molecular marker 100 pb (Invitrogen®), 0.8 µg; 2- Amplicon obtained by PCR (*EC20* synthetic gene) using as template the plasmid PGEM-EC20 and the primers T3 and T7 (Promega®); 3- *EC20* synthetic gene amplicon digested with *Bam*HI and *Hind*III; 4- *EC20* synthetic gene amplicon digested with *Bam*HI and *EcoRI*. **(d)** 1- Molecular marker 1 kb (Invitrogen®), 0.8 µg; 2- pGEX-EC20 digested with *Bam*HI and *Eco*RI; 3- Molecular Marker 100 bp DNA (Invitrogen®), 0.8 µg.

The synthetic phytochelatin EC20 expression was analyzed by SDS-PAGE. The clones BL21/pTE-EC20 expressed a 4.6 kDa protein, the corresponding expected weight for the EC20 protein (data not show). The clone BL21/pGEX-EC20 expressed a protein band with 30.6 kDa corresponding to the fusion protein EC20-GST (4.6 kDa / EC20 plus 26 kDa / GST) (Figure 2-b).

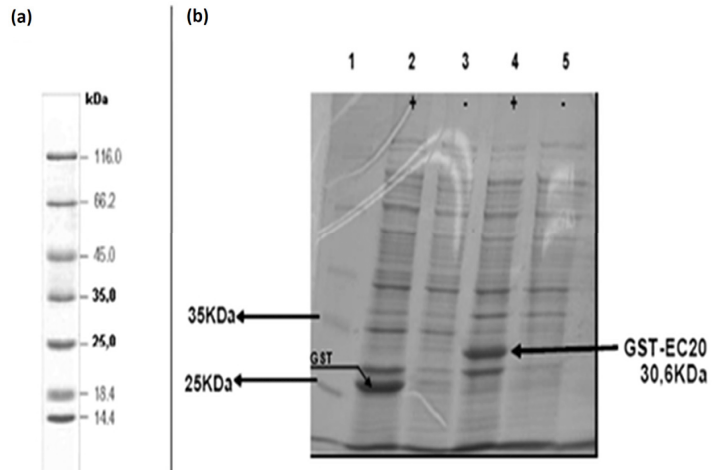

Figure 2. Expression analysis of the fusion protein EC20-GST by SDS-PAGE (17.5% acrylamide): **(a)** Protein molecular weight standard SM043 (Fermentas®). **(b)** 1- Molecular weight standard SM0431 protein (Fermentas®); 2 and 3- total proteins expressed by the negative control BL21(DE3)/pGEX-4T-2; 4 and 5- total proteins expressed by the recombinant clone BL21/pGEX-EC20: (2, 4) after growth in the induction condition of EC20 expression (2.0 mM IPTG) and (3, 5) of EC20 repression expression (with glucose).

The *E. coli* recombinant clones resistance (or tolerance) to heavy metal were analyzed by determination of the MIC (minimum inhibitory concentration) of Cd^{2+} for the clones. The MICs of Cd^{2+} for the recombinant clones came from *E. coli* DH5α or DH10B strains harboring the plasmids pGEM-EC20 (data not show) or pBsKS-EC20, the results were 2.5 mM and 0.312 mM after cells

growth, respectively, under the inducing and the repressing condition of EC20 expression, compared to 0.156 mM for the corresponding untransformed *E. coli* strains that do not express the EC20 protein (negative controls) (Figure 3-a).

The Cd^{2+} MICs for the recombinant clones derived from *E. coli* BL31 harboring the plasmid pTE-EC20 or pGEX-EC20 were 10 mM and 2.5 mM after growing the cells, respectively, under condition of induction and repression of EC20 expression; compared with 1.25 mM for the *E. coli* BL31 strain which does not express the EC20 protein (negative control) (Figure 3-b).

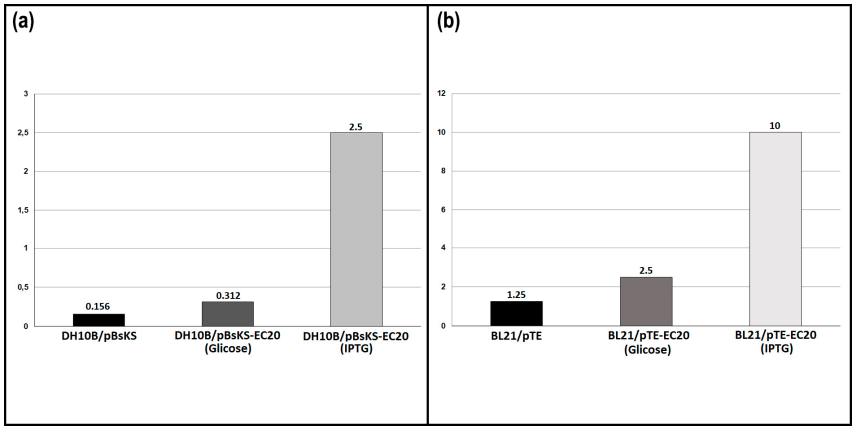

Figure 3. MIC of Cd^{2+} showed by the *E. coli* recombinant clones.
The cells were grown in MJS liquid medium supplemented with $CdCl_2$, 37 ºC, with agitation in shaker: **(a)** Recombinant clones: DH10B/pBsKS (negative controls without EC20), DH10B/pBsKS-EC20 cultured with glucose (repressed condition of EC20 expression), DH10B/pBsKS-EC20 cultured with IPTG (induction condition of EC20 expression). **(b)** Recombinant clones: BL21/pTE (negative controls without EC20), BL21/pTE-EC20 cultured with glucose (repressed condition for EC20 expression), BL21/pTE-EC20 cultured with IPTG (induction condition for EC20 expression).

The heavy metal biosorption capacity of the recombinant *E. coli* clones were settled. Cells from the bacterial recombinant clones were incubated in aqueous solution of Cd^{2+} and, after removing the cells from the solutions, the amount of remaining Cd^{2+} was quantified by ICP-AES (Inductively Coupled Plasma - Atomic Emission Spectroscopy).

The Recombinant clone BL21/pGEX-EC20 cells, after growth in the EC20 protein expression repression condition, absorbed 11.5% of the total amount of Cd^{2+} present in water; and these cells, after growth in the induction condition of the EC20 expression, absorbed 37.5% of the total amount of Cd^{2+} present in water (Figure 4).

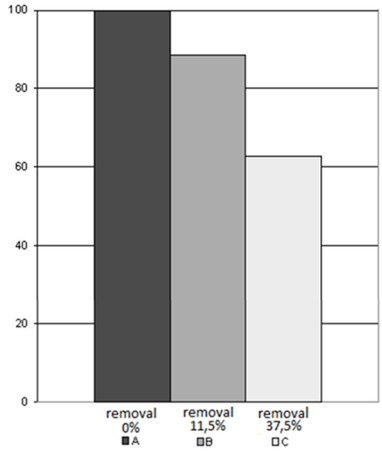

Figure 4. Absorption capacity of Cd^{2+} from *E. coli* recombinant clones.
A 1.000 µM $CdCl_2$ water solution was incubated with bacterial cells. After the treatment, the total amount of Cd^{2+} was determined by ICP ICP-AES. **A**- Untreated solution and **B**- treated solution with: BL21/pGEX-EC20 clone cells grown on glucose-medium (repressed condition for EC20 expression); and **C**- treated solution with: BL21/pGEX-EC20 clone cells grown on medium supplemented with IPTG (induced condition for EC20 expression).

DISCUSSION

In this study, the construction of recombinant *E. coli* clones expressing the EC20 synthetic phytochelatin was demonstrated, consequently, we could notice the clones increasement in their capacities for Cd^{2+} biosorption and resistance to this toxic ion.

To accomplish that, a double-stranded synthetic DNA fragment encoding for the EC20 synthetic phytochelatin (*EC20* gene) was constructed *in vitro* (Figure 1-a), amplified by PCR,inserted into the cloning plasmids (pBluescript®KS^+ and pGEM®-TEasy) and into the expression plasmids pET-28a(+)® [derivative without His-tag constructed in this work] and pGEX-4T-2®.

The molecular constructions (pGEM-EC20, pBsKS-EC20, pTE-EC20 and pGEX-EC20) were confirmed by restriction analysis, amplification by PCR (Figure 1-b, c, d) and DNA sequencing. The recombinant plasmids pGEM-EC20, pBsKS-EC20 were inserted into *E. coli* DH5α and DH10B strains. The recombinant plasmids pTE-EC20 and pGEX-EC20 were inserted into *E. coli* BL21-DE3 strain, as it offers valuable benefits of maximal expression of a cloned gene.

As expected, the recombinant clone BL21/pGEX-EC20, after growth in the inducing condition of *EC20* expression, produced a 30.6 kDa recombinant protein corresponding to a fusion of glutathione-S-transferase (GST, 26 kDa) and EC20 synthetic phytochelatin (4.6 kDa), as shown in SDS-PAGE analysis (Figure 2-b).

We were able to observe that the expression of EC20 also grants a great increasement on the resistance to Cd^{2+} of the recombinant clones. Clones harboring plasmids pBsKS-EC20 or pGEM-EC20, after growth in the condition of *EC20* expression induction, became 16 times more resistant to Cd^{2+}, and even after growth in the condition of *EC20* repressed expression, they remain 8 times more resistant to Cd^{2+} in comparison with the original untransformed strains (Figure 3-a). The clones *E. coli* BL21 harboring the plasmid pTE-EC20 or pGEX-EC20 (BL21/pTE-EC20 and BL21/pGEX-EC20), after growth in the condition of induction and repression, *EC20* expression showed, respectively, MICs to Cd^{2+} 8 and 2 times higher than the MIC of the original BL21 strain (without *EC20*) (Figure 3-b). The observed increasement on resistance to Cd^{2+} showed by the clones expressing EC20 is particularly relevant because it is a desired and valuable phenotype for a bacterium that must perform bioaccumulation of toxic heavy metal as a strategy for environmental bioremediation.

As far as we know, the only previous study describing increasement to metal resistance resulting from EC20 expression was seen in *D. radiodurans*, and in that case, the 2.5-fold increasement was considered an amazing result (CHATURVEDI; ARCHANA, 2014).

The ICP-AES analysis was used to quantify the remaining amount of Cd^{2+} present in water after treatment with a 1.000 μM Cd^{2+} solution with bacterial cells. Cells of the recombinant clone BL21/pGEX-EC20, after growth in the condition of induction of EC20 protein expression, showed capacity for removing 37.5% of the total amount of Cd^{2+} present in that solution and, even when these cells were grown in the condition of EC20 protein expression repression, they removed 11.5% of the total amount of Cd^{2+} (Figure 4). So, induction of EC20 protein expression promotes 26% increasement in Cd^{2+} bioaccumulation of the recombinant clone cells (Figure 4).

These are satisfactory results that can be comparable to those previously described with recombinant clones expressing EC20 (BAE et al., 2000), *C. metallidurans* (BIONDO et al., 2012) and *D. radiodurans* (CHATURVEDI; ARCHANA, 2014). This indicates that this approach offers a good potential for the construction of new bacterial strains useful for bioremediation of wastewaters containing heavy metals or to recover valuable metals that still remains in those waters.

CONCLUSION

It was successfully describe the construction and characterization of recombinant *E. coli* clones expressing intracellularly the synthetic phytochelatin EC20. As expected, the recombinant clones, in comparison to untransformed cells, showed an increased biosorption capacities of Cd^{2+}, and this confirms that this approach offers good and new prospects for future applications in bioremediation procedures of water contaminated with heavy metals as cadmium or even for recovery of valuable heavy metals present in water. Moreover, in this research, it was demonstrated that EC20 expression also promotes an increasement on the bacterial resistance to Cd^{2+}.

RESUMO: Foram construídos clones recombinantes de *E. coli* que expressam intracelularmente a fitoquelatina sintética EC20. Foi analisado o aumento na capacidade de biossorção de Cd^{2+} e o aumento da resistência a este metal tóxico. Foi sintetizado *in vitro* um gene codificante da fitoquelatina sintética EC20. O gene *EC20* sintético foi amplificado por PCR, inserido nos vetores de clonagem pBluescript®KS^+ e pGEM®-TEasy, e nos vetores de expressão pTE [derivado de pET-28(a)®] e pGEX-T4-2®. Os plasmídeos recombinantes foram empregados na transformação genética de *E. coli*: pBsKS-EC20 e pGEM-EC20 foram introduzidos nas linhagens DH10B e DH5α; e, pTE-EC20 e pGEX-EC20 na linhagem BL21-DE3. A expressão EC20 foi analisada por SDS-PAGE. As resistências a Cd^{2+} dos clones recombinantes foram determinadas por análises de MIC. A MIC para Cd^{2+} de DH10B/pBsKS-EC20 e de DH10B/pGEM-EC20 foi 2,5 mM (*EC20* induzido) e 0,312 mM (*EC20* reprimido); respectivamente, 16 e 2 vezes superiores às do controle DH10B/pBsKS (0,156 mM). A MIC para Cd^{2+} de BL21/pTE-EC20 foi 10,0 mM (*EC20* induzido) e 2,5 mM (*EC20* reprimido), comparado a do controle BL21/pTE que foi apenas 1,25 mM. A análise de ICP-AES mostrou que BL21/pGEX-EC20, após crescimento na condição de expressão de EC20, absorveu 37,5% de Cd^{2+} e, mesmo quando cultivado na condição de não-indução de expressão EC20, absorveu 11,5% de Cd^{2+}. Estes resultados permitem a conclusão de que os clones recombinantes de *E. coli* que expressam a fitoquelatina sintética EC20 apresentam aumento da capacidade de biossorção de Cd^{2+} e de resistência a este íon tóxico.

PALAVRAS-CHAVES: *Escherichia coli*. Fitoquelatina. Biossorção. Biorremediação. Cádmio.

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