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Carbon Storage Regulator A (*csrA*) Gene Regulates Motility and Growth of *Bacillus licheniformis* in the Presence of Hydrocarbons

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The global carbon storage regulator (Csr) system is conserved in bacteria and functions as a regulator in the exponential and stationary phases of growth in batch culture. The Csr system plays a role in the central carbon metabolism, virulence, motility, resistance to oxidative stress, and biofilm formation. Although the Csr was extensively studied in Gram negative bacteria, it has been reported only in the control of motility in *Bacillus subtilis* among Gram positive bacteria. The goal of this study was to explore the role of the *csrA* gene of *Bacillus licheniformis* M2-7 on motility and the bacterial ability to use hydrocarbons as carbon source. We deleted the *csrA* gene of *B. licheniformis* M2-7 using the plasmid pCsr-L, harboring the spectinomycin cassette obtained from the plasmid pHP45-omega2. Mutants were grown on culture medium supplemented with 2% glucose or 0.1% gasoline and motility was assessed by electron microscopy. We observed that *CsrA* negatively regulates motility by controlling the expression of the *hag* gene and the synthesis of flagellin. Notably, we showed the ability of *B. licheniformis* to use gasoline as a unique carbon source. Our results demonstrated that *CsrA* is an indispensable regulator for the growth of *B. licheniformis* M2-7 on gasoline.

Keywords: **Csr system, regulation, *Bacillus licheniformis,* motility, growth, hydrocarbons**

Introduction

Carbon storage regulator (Csr) is a global regulatory system that controls bacterial gene expression post-transcriptionally, and is constituted by two fundamental units, CsrA and CsrB [1]. The protein CsrA is a central component, having a close relationship with mRNA binding proteins [2, 3]. By the contrary, CsrB is a non-translatable RNA molecule of 360 base pairs, which has a structure of stems and handles [1].

In the mechanism of regulation mediated by Csr system, the main role of CsrA is the negative regulation of translation, since it has the ability to bind to the mRNAs, recognizing the consensus sequence 5'RUACARGGAUGU3'. These RNA sites are consistent with the Shine-Dalgarno ribosome binding site (SD) of its target mRNAs, thus preventing the binding of ribosomes, and repressing translation [4, 5]. CsrB is a small non-coding

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RNA molecule that binds to CsrA, unblocking the SD site and antagonizing its repressor activity [1].

The homologous system to Csr in non-enteric bacteria is the repressor secondary metabolism system (Rsm), which is also constituted by an mRNA binding protein (RsmA) and at least one non-translatable RNA molecule (RsmB) with the ability to bind to RsmA. The Csr or Rsm system has been widely studied in *E. coli* and reported in several Gram negative bacteria, such as *Erwinia carotovora, Pseudomonas aeruginosa, Salmonella enterica, Vibrio cholerae, Acinetobacter baumannii, Azotobacter vinelandii, Serratia marcescens* and Gram positive bacteria such as *Bacillus subtilis* [6, 7] and *Clostridium acetobutylicum* [8]. Csr is considered a global regulation system because is widespread in Gram negative bacteria, controlling functions, such as carbon storage, mobility, quorum sensing, virulence, biofilm formation, and production of polymers [1, 9–11].

However, in Gram positive bacteria, it has been reported only in *C. acetobutylicum* and *C. difficile* [8, 12, 13]. In *B. subtilis* CsrA regulates the synthesis of flagellin by inhibiting the hag gene translation, although the antagonist is the protein Fliw [12]. In *C. acetobutylicum,* CsrA is involved in flagellum regulation, central carbon metabolism, sporulation and the control of transport systems such as iron, oligopeptides and the phosphotransferase system [8]. In *C. difficile,* it also controls multiple virulence processes including toxin production, motility and adhesion, as well as in carbon metabolism [13].

B. licheniformis is recognized by multiple biotechnological applications such as: 1) bioremediation of sites contaminated by pesticides, fertilizers, hydrocarbons and heavy metals, 2) biological control in the development of biofungicides due to antifungal activity, 3) concrete production for its ability to precipitate calcium carbonate, and 4) biofertilizers for the characteristics of plant growth promotion [14]. *B. licheniformis* M2-7, is a mobile, facultative anaerobic, and thermoresistant bacterium isolated from hot springs [15]. Its growth temperature ranges from 37 to 60℃, although it can resist up to 110℃. Also, it has a chemoganotropic metabolism capable of using various carbohydrates such as glucose, maltose, sucrose and lactose (Unpublished data). We showed that the M2-7 strain is capable of degrading petroleum hydrocarbons, gasoline, diesel and burnt oil. In addition, it can grow in the presence of polycyclic aromatic hydrocarbons (PAHs) such as naphthalene, phenanthrene, pyrene and benzo [a] pyrene, demonstrating that it can biotransform benzo [a] pyrene to talic acid [15]. A preliminary *in silico* analysis of the *B. licheniformis* genome revealed the presence of genes homologous to *csrA* and *fliW* with a genomic arrangement similar to that of *B. subtilis*. However, the role of the CsrA-FliW system in *B. licheniformis* has not been reported; consequently, the relationship of this system with the hydrocarbon degradation capacity of the bacteria has been elucidated. Here we explored the effect of inactivation of *CsrA* on mobility and examined its effect on growth in the presence of gasoline as carbon source in *B. licheniformis* M2-7. The ability of csrA-inactivated LYA-5 to grwon in the presence of gasolin allows the study on sites contaminated with hydrocarbons, to generate a bioremediation strategy for these recalcitrant pollutants.

Materials and Methods

Bacterial strains and growth conditions

The strains, plasmids and oligonucleotides used in this study are listed in Table 1. Strains of *B. licheniformis* M2-7 and *E. coli* DH5α, were grown in Luria Bertani medium (LB) with a composition in g/l: peptone of casein 10, sodium chloride 10, yeast extract 5 for liquid medium and supplemented with 15 g/l of agar for solid medium) at 37℃. The concentration of spectinomycin (Sp, μg/ml) used for *B. licheniformis* M2-7 and *E. coli* DH5α was 200 and 100, respectively. For competent cells of *E. coli* DH5α preparation, the method described by Sambrook and Russell [16] was used. For *Bacillus licheniformis* M2-7, they were prepared according to the methodology of Hoffmann *et al.* [17]. For growth assays Bushnell-Haas culture medium (BH) supplemented with 2% glucose or 0.1% gasoline was used. We used gasoline since in previous studies it was where we observed a better growth of the M2-7 strain than in diesel or oil (data not shown).

Construction of the plasmid pCsr-L and the mutant strain of *Bacillus licheniformis* LYA-5

To inactivate the *csrA* gene of *B. licheniformis* M2-7, the plasmid pCsr-L was constructed (Table 1), which was generated from the insertion of the spectinomycin cassette, obtained from the cut of the plasmid pHP45-

Table 1. Strains, plasmids and oligonucleotides that were used in this work.

Strain	Description	References
B . licheniformis M2-7	Filamentous, flat, beige colony, with a diameter of 0.7 mm, chemoganotrofa, Oxidase (+), VP (+) and C. nitrate (+), Sp^S.	Unpublished data
LYA-5	M2-7 *csrA*::*Sp*,Sp^R	This work
E. coli DH5α	*supE44, ΔlacU169, hsdR17, recA1, endA1, gyrA96, thi-1 relA1*	Hanahan (1983)
Plasmids		
pJET1.2/blunt	Plasmid to clone PCR products	Thermo Fisher scientific (2015)
pJIER	Plasmid derived from pJET containing the *csrA* gene from *B. licheniformis* M2-7, Ap^R	Unpublished data
pHP45-omega2	Plasmid with the spectinomycin resistance cassette Sp^R	Joachim *et al.* (1987)
pCsr-L	Plasmid derived from pJIER containing *csrA*:: Sp, Sp^R, Ap^R	This work
Oligonucleotides	Sequence	
F*Sp*	$^{5'}$GGACAAATTCTTCCAACTG$^{3'}$	This work
R*Sp*	$^{5'}$GCAATTTGGAGAATGGCA$^{3'}$	This work
FD16´S	$^{5'}$AGAGTTTGATCCTGGCTCAG$^{3'}$	This work
RD16´S	$^{5'}$AAGGAGGTGATCCAGCC$^{3'}$	This work
F1-$csrA_{700pb}$	$^{5'}$GTGTTAAGCGCTGCAATG$^{3'}$	This work
R1-$csrA_{700pb}$	$^{5'}$TGCCGCTTTCCGAGGATT$^{3'}$	This work
F1-*gyrA*	$^{5'}$ACACAGAAGCGAGAATGTCTAAA$^{3'}$	This work
R1-*gyrA*	$^{5'}$CCGTCATAGTTGTCCTGGTAATC$^{3'}$	This work
F1-*csrA*	$^{5'}$GTTGACGGAGATCAGGTGAAA$^{3'}$	This work
R1-*csrA*	$^{5'}$AAGCAAATCGTTTGAGATGGAAG$^{3'}$	This work
F1-*hag*	$^{5'}$CGAACACGTTGAAGGATTGAG$^{3'}$	This work
R1-*hag*	$^{5'}$TTCTAAGAACGCTCAAGACGG$^{3'}$	This work

omega2 with the enzyme *SmaI*, in the pJIER plasmid linearized with the restriction enzyme *EcoRV* and ligated with the T4 DNA ligase (Thermoscientific®). The generation of the pCsr-L plasmid was confirmed by PCR using plasmid DNA and the oligonucleotides FSp and RSp and by restriction reaction with the *HindIII* enzymes. Once the plasmid pCsr-L was generated, competent *Bacillus licheniformis* M2-7 cells were transformed and the transformant cells resistant to spectinomycin were selected. The deletion of the *csrA* gene by double recombination was confirmed by PCR using genomic DNA of the recombinant strain with the oligonucleotides FSp and RSp and FcsrA and RcsrA. Amplification control was carried out using genomic DNA from the wild strain *Bacillus licheniform*is M2-7 and LYA-5 and the oligonucleotides FD16'S and RD16'S for the amplification of the 16S rRNA gene.

Motility assays and electron microscopy

Motility was examined by inoculating 1 μl of a 16-hour bacterial culture on to 0.9% LB agar plates. They were incubated at 37℃ for 24 h [18]. For electron microscopy the strains were grown in solid LB medium at 37℃ for 24 h. A colony was printed and resuspended in 10 μl of water, then the cells were stained with uranyl acetate and lead in the citrate and observed with a ZEISS Libra 120 plus electron microscope.

Evaluation of the growth of *B. licheniformis* M2-7 and LYA-5 in glucose and hydrocarbons as a carbon source

The growth of strain M2-7 was evaluated by inoculating 1 ml of a 12-hour culture in 30 ml in Bushnell-Haas medium supplemented with 2% glucose or with 0.1% gasoline, remaining at a final D.O. 600 nm of 0.5. The gasoline (87 octane) was filtered using a Millipore brand nitrocellulose membrane. It was incubated at 37℃ with shaking at 200 rpm for 6, 12, 24 and 36 h under aerobic conditions. As a negative control, BH medium without carbon source was used, inoculated with *B. licheniformis* M2-7, as well as BH supplemented with the carbon source without inoculum. Strain *B. licheniformis* LYA-5 was grown under the same conditions mentioned above.

Protein quantification was performed by the method of Lowry *et al.* [19].

Expression of the *csrA* and hag genes by Real Time PCR

To quantify the level of expression of the *csrA* and *hag* genes, the extraction and purification of the total RNA of the strain of *Bacillus licheniformis* M2-7 and LYA-5 were carried out, following the protocol described by Rojas-Aparicio *et al.* [20] with some modifications. The contaminating residual DNA was treated with DNase I (Thermo Scientific, USA) and the RNA was stored at -70℃. For the generation of complementary DNA (cDNA) by reverse transcription, the RevertAid kit (Thermo Scientific) was used. The reaction was performed as follows: 1 µg of total RNA, 20 pmol/µl of the reverse oligonucleotides (Table 1), 2 µl of dNTP's (10 mM), 4 µl of 5X reaction buffer, 1 µl of RNase inhibitor (40 U) and 0.5 µl of reverse transcriptase. The reaction was incubated at 42℃ for 1 h, inactivated at 70℃ for 10 min and the cDNA was stored at -20℃. The obtained cDNA was used as a template for the qRT-PCR reaction, the oligonucleotides used are listed in Table 1. The gyrA gene was used as an endogenous control. The qRT-PCR was performed on a CFX96 Touch™. Each reaction contained: 1 µl of cDNA, 10 µl of Master Mix 2x (LightCycler® 480 SYBR Green I, from Roche, Switzerland) and 2.5 mM of oligonucleotide mixture (Table 1). Each reaction was performed in triplicate. The amplification conditions were performed as follows: denaturation at 95℃ for 10 min, followed by 40 cycles at 95℃ for 10 sec and 60℃ for 60 sec. The analysis of the relative expression was carried out using the 2-ΔΔCT method [21].

Statistical analysis

All experiments were performed in triplicate at different times. The GraphPad Prism5 software was used to create graphics. For the Statistical Analysis ANOVA was performed, for independent samples ($p \leq 0.05$), and the Student t test with a value of $p \leq 0.05$ was considered significant.

Results

CsrA regulates the growth of *Bacillus licheniformis* M2-7 in the presence of glucose and hydrocarbons

To determine if the CsrA regulator has a role in the growth of *B. licheniformis* on hydrocarbons, the strain LYA-5 was constructed in which the *csrA* gene was inactivated. The growth of *B. licheniformis* M2-7 and *B. licheniformis* LYA-5 were compared in BH medium supplemented with 2% glucose. Both strains showed similar growth patterns from 6 to 36 h of growth (Fig. 1).

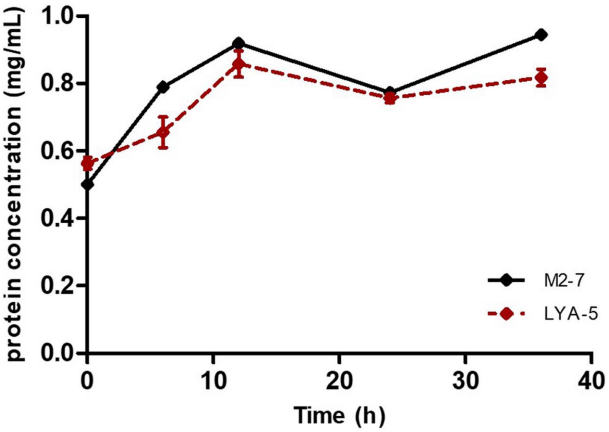

Fig. 1. Growth kinetics in the presence of 2% glucose. Quantification of protein from the wild strain (•) and mutant strain (◆) at times 0, 6, 12, 24 and 36 h in Bushnell-Haas at 37℃. The values are shown as the average of three replicates.

Subsequently, the growth of strains M2-7 and LYA-5 were analyzed in BH medium supplemented with 0.1% gasoline. Fig. 2 shows the growth kinetics. The wild strain M2-7 had a slight growth during the first 12 h of

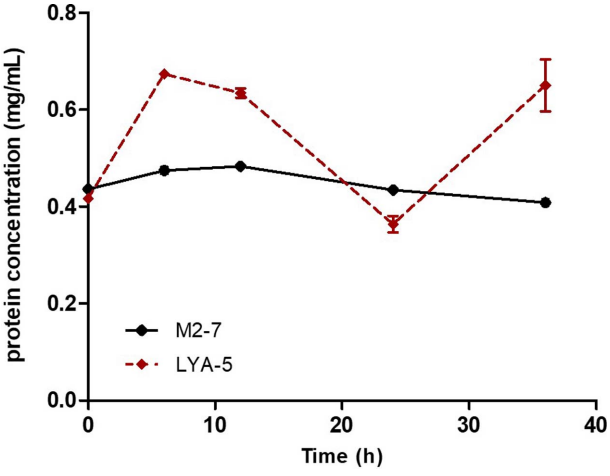

Fig. 2. *Bacillus licheniformis* kinetics in Busnhell-hass medium supplemented with 0.1% gasoline. Protein quantification from wild (•) and mutant (◆) strains at 0, 6, 12, 24 and 36 h in Bushnell-Haas at 37℃. The values are shown as the average of three replicates and data were analyzed with two-way ANOVA obtaining a value of $p \leq 0.05$

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incubation and subsequently decreased, while the growth of the strain LYA-5 was higher than that of the wild strain. These results suggest that CsrA regulates the ability to grow in the presence of hydrocarbons as a carbon source in *B. licheniformis*.

The expression of *csrA* increases in the presence of hydrocarbons with respect to the glucose carbon source in *Bacillus licheniformis*

In order to know the relative levels of *csrA* transcription in the different carbon sources, expression was analyzed in cultures in presence of 2% glucose or 0.1% gasoline as carbon sources at 6, 12 and 24 h. In the M2-7 strain, the levels of *csrA* increased two times in the first six hours in gasoline, while at 12 h 29 times more with respect to the source of carbon glucose. Interestingly, after 24 h of incubation, expression of *csrA* decreased considerably in both conditions (Fig. 3). We corroborated that the expression of *csrA* in the mutant LYA-5 decreased, observing that in the strain LYA-5 the expression at 6 h decreased 124.8 times in BH medium supplemented with glucose and 2,087.88 times in BH medium supplemented with gasoline with respect to the wild strain (data not shown). Taken together, these results suggest that the expression of *csrA* is differential during the growth of *B. licheniformis* M2-7, and that CsrA is involved in regulation to promote growth on gasoline as a carbon source.

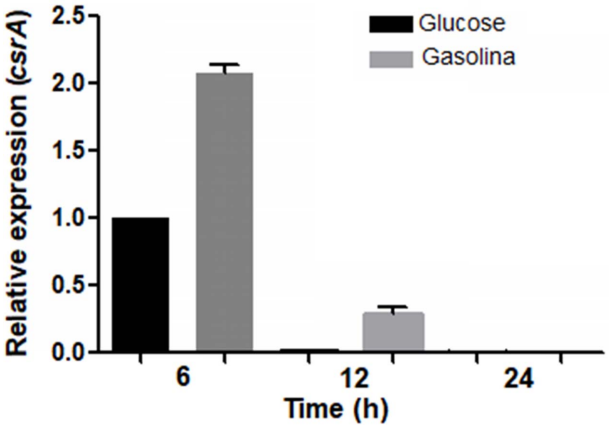

Fig. 3. Expression of *csrA* of *Bacillus licheniformis* M2-7. Expression at times 6, 12 and 24 h in BH medium supplemented with 2% glucose (■) or 0.1% gasoline (■). Error bars are the standard deviations of three replicates.

CsrA regulates motility in *Bacillus licheniformis*

To determine whether CsrA influenced cell motility in *B. licheniformis*, as in *B. subtilis*, the motility of strain M2-7 and strain LYA-5 were analyzed on 0.9% LB agar plates. The results showed that strain LYA-5 exhibited greater mobility compared with the wild strain (Fig. 4A). When comparing the morphology of the two strains by electron microscopy (Fig. 4B), a morphological difference was observed between both strains. We performed Gram stain on bacteria in both conditions to rule out contamination (Fig. 4C). While the wild strain showed a flagellation of amphitic type with few flagella, strain LYA-5 showed a perimeter flagellation. When comparing the levels of transcription of the *hag* gene between the two strains, it was observed that in strain LYA-5 (Fig. 5), these were significantly higher than in the wild strain (2.5 times). All results together show that CsrA is involved in the regulation of flagellum synthesis in *Bacillus licheniformis*.

Discussion

Bacterial adaptation is crucial for survival in the environment and depends on a broad genes regulation [1, 22]. In addition, a variety of transcriptional and post-transcriptional mechanisms are often interconnected, underlying these global responses. The Csr system is one of the mechanisms that has great influence on a variety of physiological processes such as central carbon metabolism, biofilm formation, motility, peptide uptake, virulence and pathogenesis, quorum detection and response to oxidative stress [1, 9–11]. In this system, the main effector is CsrA, a protein that acts as a posttranscriptional regulator [23]. Through an in silico analysis, we identified the presence of csrA and fliW in the genome of *B. lichenifirmis*. With this information, the experiments started to determine if the CsrA regulator played a role in the growth on hydrocarbons. First, we observed that both strains showed similar growth patterns from 6 to 36 h of growth (Fig. 1). The inhibition of *csrA* did not affect the growth of the bacteria in this carbon source, while in BH medium supplemented with 0.1% gasoline CsrA plays an important role, since the mutant strain LYA-5 showed greater growth than the wild strain. It should be mentioned that there is not much data reported about the participation of *csrA* in

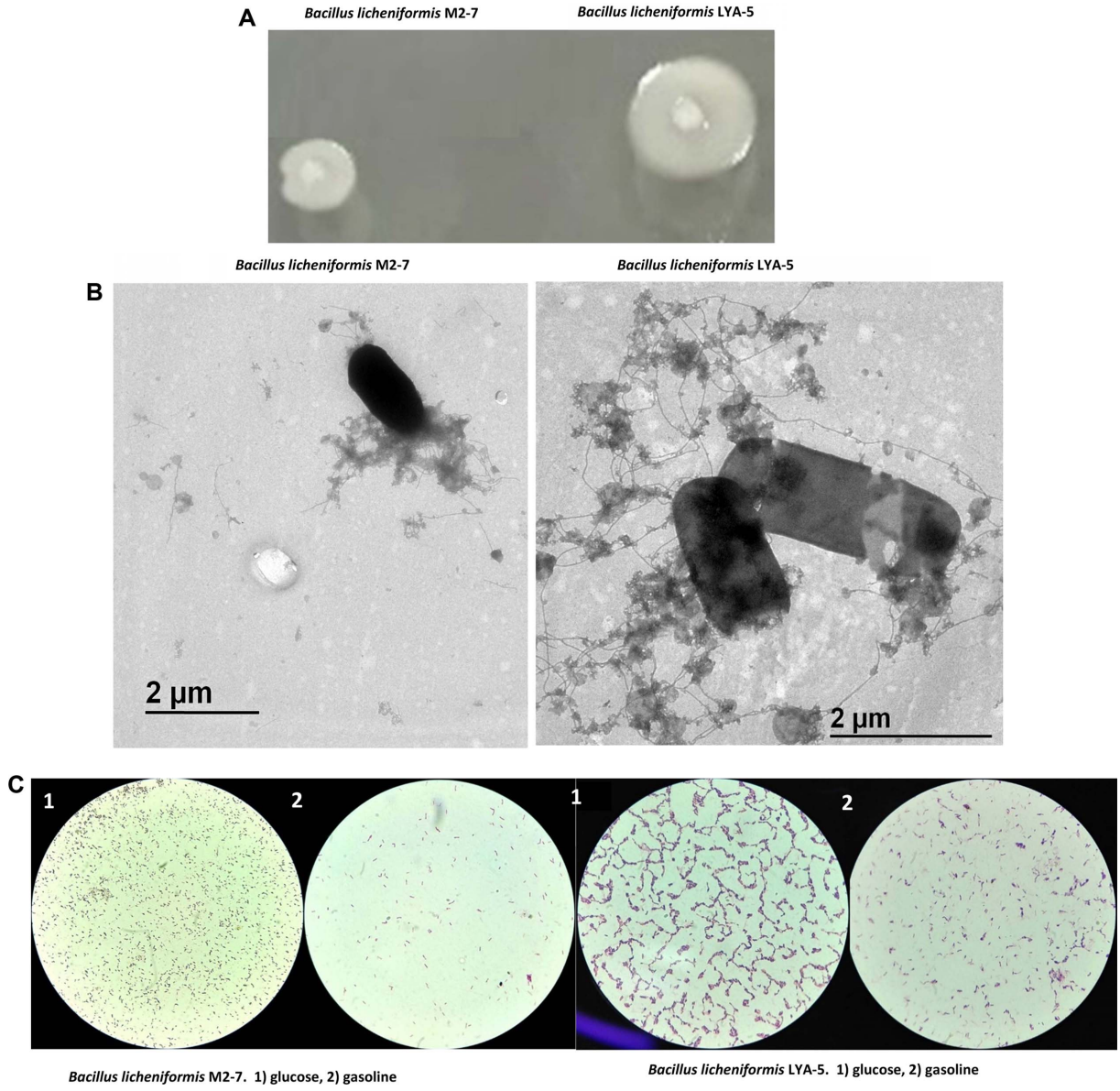

Fig. 4. Effect of the inactivation of *csrA* on the motility and flagellin synthesis in *Bacillus licheniformis*. (A) Swim test on LB agar plates at 9.0%, (B) Electron microscopy of *Bacillus licheniformis* strains, (C) Gram stain.

Gram positive bacteria, since most of the investigations have been carried out in Gram negative bacteria, with *E. coli* being the main study model, where *csrA* is an activator of glycolysis and a repressor of gluconeogenesis [1]. In this research work, the relative transcription levels of the *csrA* gene, in *B. licheniformis* M2-7, were measured in glucose and gasoline as a carbon source, observing that the expression of *csrA* was differential during growth in both conditions. We also observed that the transcription of *csrA* is increased twice as much in the presence of 0.1% gasoline as in the presence of glucose as a carbon source (Fig. 3). During the transition from exponential growth to the stationary phase, the bacteria readjusted their physiological state on a large scale, from one that allows growth, to another that provides greater resistance to stress and an improved capacity to eliminate the substrate from the medium [24, 25]. *csrA* activates certain metabolic pathways in the exponential phase and represses numerous genes whose expression occurs in the stationary phase of growth [1]. Therefore, it

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is deduced that *csrA* may act as a regulator in the synthesis of various enzymes that allow *B. licheniformis* M2-7 to grow in the presence of hydrocarbons through the activation of metabolic pathways or through the synthesis of enzymes that provide greater resistance to stress, affecting the enzymes involved in the exponential and stationary phase of growth.

In our knowledge, this is the first evidence about the role of *csrA* expression under a recalcitrant contaminant. Rojas-Aparicio *et al.* [20] reported that *B. licheniformis* M2-7 in the presence of polycyclic aromatic hydrocarbons increases the expression of the *catE*, *pobA* and *fabHB* genes, whose products are proteins related to the metabolism of hydrocarbons [20], therefore, the Csr system could be regulating growth on gasoline by controlling the expression of these genes. In this study, we also evaluated the role of CsrA on motility of *B. licheniformis*. This protein coordinated the expression of flagellin, because in the LYA-5 strain, *hag* expression was increased (Fig. 5), which agrees with that described by Mukherjee *et al.* [26], where they observed that the inhibition of the *csrA* gene causes mutant strains to have greater motility in *B. subtilis*. The result was corroborated with an electron micrograph, observing that the wild-type strain presented an amphitic flagellation, while the mutant strain showed a perimeter flagellation (Fig. 4B), indicating an increase in the amount of flagellin in the mutant strain comapred to the wild type strain. The role of CsrA in motility (regulation of *flhDC* genes) and in cell morphology has been reported in many Gram negative bacteria [3, 27]. With regard to Gram positive bacteria, there are few reports on the characterization of this system on motility. For example in *B. subtilis,* there is an increase of *hag* gene expression after inhibition of csrA [26]. In *C. acetobutylicum*, *csrA* is involved in the regulation of the assembly of flagella [8], whereas in *C. difficile,* it has been reported that the overexpression of *csrA* resulted in a defect in the flagella generating a decrease in motility [13]. CsrA is a highly conserved RNA binding protein whose main role could be the regulation of flagellar assembly, suggesting that it is performing the same function in *B. licheniformis*, since the *csrA* gene shows a remarkable phylogenetic distribution and is restricted mainly to those bacterial genomes encoding flagellin [28]. Altogether, results provide new knowledge about Csr system in Gram positive bacteria and allow to understand the CsrA role in heat-resistant bacteria with a greater capacity to degrade hydrocarbons, which could be used for bioremediation.

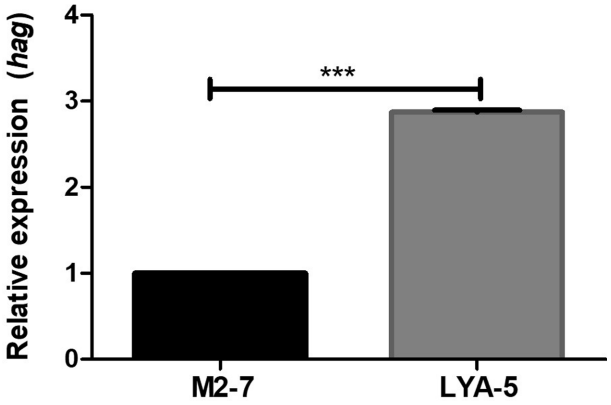

Fig. 5. Expression of *hag* in *Bacillus licheniformis* M2-7 and *Bacillus licheniformis* LYA-5 at 6 h. The values are the average of three independent experiments; Error bars, SD. ***denotes the value of the Student's t test with a value of $p \leq 0.05$.

Acknowledgments

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Romeo T. 1998. Global regulation by the small RNA-binding protein CsrA and the non-coding RNA molecule CsrB. *Mol. Microbiol.* **29**: 1321-1330.
2. Babitzke P, Romeo T. 2007. CsrB sRNA family: sequestration of RNA-binding regulatory proteins. *Curr. Opin. Microbiol.* **10**: 156-163.
3. Liu MY, Gui G, Wei B, Preston JF 3er, Oakford L, Yüksel U, *et al.* 1997. The RNA molecule CsrB binds to the global regulatory protein CsrA and antagonizes its activity in *Escherichia coli*. *J. Biol. Chem*. **272**: 17502-17510.
4. Suzuki K, Wang X, Weilbacher T, Pernestig AK, Melefors O, Georgellis D, *et al.* 2002. Regulatory circuitry of the CsrA/CsrB and BarA/UvrY systems of *Escherichia coli*. *J. Bacteriol.* **184**: 5130-5140.
5. Dubey AK, Baker CS, Romeo T, Babitzke P. 2005. RNA sequence

and secondary structure participate in high-affinity CsrA-RNA interaction. *RNA* **11**: 1579-1587.
6. Lapouge K, Schubert M, Allain FH, Haas D. 2008. Gac/Rsm signal transduction pathway of gamma-proteobacteria: from RNA recognition to regulation of social behaviour. *Mol. Microbiol*. **67**: 241-253.
7. Romero Y, Guzman J, Moreno S, Cocotl-Yanez M, Vences-Guzman MA, Castaneda M, *et al*. 2016. The GacS/A-RsmA signal transduction pathway controls the synthesis of Alkylresorcinol Lipids that replace membrane phospholipids during encystment of *Azotobacter vinelandii* SW136. *PLoS One* **11**: e0153266.
8. Tan Y, Liu ZY, Liu Z, Zheng HJ, Li FL. 2015. Comparative transcriptome analysis between *csr*A-disruption *Clostridium acetobutylicum* and its parent strain. *Mol. Biosyst.* **11**: 1434-1442.
9. Esquerre T, Bouvier M, Turlan C, Carpousis AJ, Girbal L, Cocaign-Bousquet M. 2016. The Csr system regulates genome-wide mRNA stability and transcription and thus gene expression in *Escherichia coli*. *Sci. Rep*. **6**: 25057.
10. Figueroa-Bossi N, Schwartz A, Guillemardet B, D'Heygere F, Bossi L, Boudvillain M. 2014. RNA remodeling by bacterial global regulator CsrA promotes Rho-dependent transcription termination. *Genes Dev.* **28**: 1239-1251.
11. Broberg M, Lee GW, Nykyri J, Lee YH, Pirhonen M, Palva ET. 2014. The global response regulator ExpA controls virulence gene expression through RsmA-mediated and RsmA-independent pathways in *Pectobacterium wasabiae* SCC3193. *Appl. Environ. Microbiol.* **80**: 1972-1984.
12. Mukherjee S, Babitzke P, Kearns DB. 2013. FliW and FliS function independently to control cytoplasmic flagellin levels in *Bacillus subtilis*. *J. Bacteriol*. **195**: 297-306.
13. Gu H, Qi H, Chen S, Shi K, Wang H, Wang J. 2018. Carbon storage regulator CsrA plays important roles in multiple virulence-associated processes of *Clostridium difficile*. *Microb. Pathog*. **121**: 303-309.
14. Toribio-Jiménez J, Rodríguez-Barrera MÁ, Hernández-Flores G, Ruvacaba-Ledezma JC, Castellanos-Escamilla M, Romero-Ramírez Y. 2017. Isolation and screening of bacteria from Zea mays plant growth promoters. *Rev. Int. Contam. Ambient* **33**: 143-150.
15. Guevara-Luna J, Alvarez-Fitz P, Rios-Leal E, Acevedo-Quiroz M, Encarnacion-Guevara S, Moreno-Godinez ME, *et al*. 2018. Biotransformation of benzoa]pyrene by the thermophilic bacterium *Bacillus licheniformis* M2-7. *World J. Microbiol. Biotechnol*. **34**: 88.
16. Sambrook J, Russell DW. 2006. Preparation and Transformation of Competent *E. coli* Using Calcium Chloride. *CSH Protoc.* **2006**: pdb.prot3932.
17. Hoffmann K, Wollherr A, Larsen M, Rachinger M, Liesegang H, Ehrenreich A, *et al.* 2010. Facilitation of direct conditional knockout of essential genes in *Bacillus licheniformis* DSM13 by comparative genetic analysis and manipulation of genetic competence. *Appl. Environ. Microbiol*. **76**: 5046-5057.
18. Shimizu K. 2013. Regulation systems of bacteria such as *Escherichia coli* in response to nutrient limitation and environmental stresses. *Metabolites* **4**: 1-35.
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. **193**: 265-275.
20. Rojas-Aparicio A, Rojas-Aparicio A, Hernández-Eligio JA, Hernández-Eligio JA, Toribio-Jiménez J, Toribio-Jiménez J, *et al.* 2018. Research article genetic expression of *pob*A and *fab*HB in *Bacillus licheniformis* M2-7 in the presence of benzo[a]pyrene. *Genet. Mol. Res*. **17**(2): gmr16039916.
21. Schmittgen TD, Livak KJ.2008. Analyzing real-time PCR data by the comparative CT method. *Nat. Protoc.* **3**: 1101-1108.
22. Gottesman S. 1984. Bacterial regulation: global regulatory networks. *Annu. Rev. Genet*. **18**: 415-441.
23. Romeo T, Gong M, Liu MY, Brun-Zinkernagel AM. 1993. Identification and molecular characterization of *csr*A, a pleiotropic gene from *Escherichia coli* that affects glycogen biosynthesis, gluconeogenesis, cell size, and surface properties. *J. Bacteriol.* **175**: 4744-4755.
24. Hengge-Aronis R. 1999. Interplay of global regulators and cell physiology in the general stress response of *Escherichia coli*. *Curr. Opin. Microbiol*. **2**: 148-152.
25. Huisman GW, Siegele DA, Zambrano MM, Kolter R. 1996. Morphological and physiological changes during stationary phase. pp. 1672-1682. *In*: R Curtiss III, FC Neidhardt, JL Ingraham, CC Lin E, KB Low, B Magasanik, *et al.* (Eds.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, American Society for Microbiology, Washington, D.C.
26. Mukherjee S, Yakhnin H, Kysela D, Sokoloski J, Babitzke P, Kearns DB. 2011. CsrA-FliW interaction governs flagellin homeostasis and a checkpoint on flagellar morphogenesis in *Bacillus subtilis*. *Mol. Microbiol.* **82**: 447-461.
27. Wei BL, Brun-Zinkernagel AM, Simecka JW, Pruss BM, Babitzke P, Romeo T. 2001. Positive regulation of motility and *flh*DC expression by the RNA-binding protein CsrA of *Escherichia coli*. *Mol. Microbiol*. **40**: 245-256.
28. Baker CS, Morozov I, Suzuki K, Romeo T, Babitzke P. 2002. CsrA regulates glycogen biosynthesis by preventing translation of *glg*C in *Escherichia coli*. *Mol. Microbiol.* **44**: 1599-1610.

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