Molecular design of *B. subtilis* strains overexpressing the *hbs* gene

Lidia Vidal[1], Lissett E. Martínez-Magaña[2], Mario Pedraza-Reyes[2*]

[1]Licence 2 Sciences de la Vie et de la Terre, Université Caen Normandie Republic of France; [2]Division of Natural and Exact Science, Department of Biology, University of Guanajuato, Guanajuato, México.

* pedrama@ugto.mx

Introduction

Bacillus subtilis, as other microorganisms, has evolved mechanisms to prevent and eliminate genomic lesions generated by oxidizing agents like hydrogen peroxide (H_2O_2). During oxidative stressful conditions, reactive oxygen species (ROS) like the superoxide and hydroxil radicals can modify the chemical structure of proteins, lipids, and nucleic acids, specially guanine creating the lesion 8-OxoG. The cytotoxic and genotoxic effects of this lesion are prevented in *B. subtilis* by the oxidized guanine (GO) system. Previous reports showed that a strain deficient in the GO system is hyper-mutagenic and hyper-resistant to H_2O_2 (1,2).

A global analyses of the proteome of a *B. subtilis* strain deficient for the GO system identified Hbs (HupA) as a downregulated factor compared to the WT strain (1). Therefore, this work was aimed to investigate if Hbs contributes to the hyperresistance to oxidative stress (HROS) and hypermutagenicity (HPM) phenotypes of the GO-deficient strain.

Results

Hbs is one of the most abundant proteins in *B. subtilis*is possessing 50,000 copies per genome in vegetative cells and spores. This protein prevents DNA denaturation under extreme environmental conditions. However, as its encoding genes is essential, the experimental approach was to overexpress the gene in the WT and GO-deficient strains.

Amplification of *hbs* gene for PCR

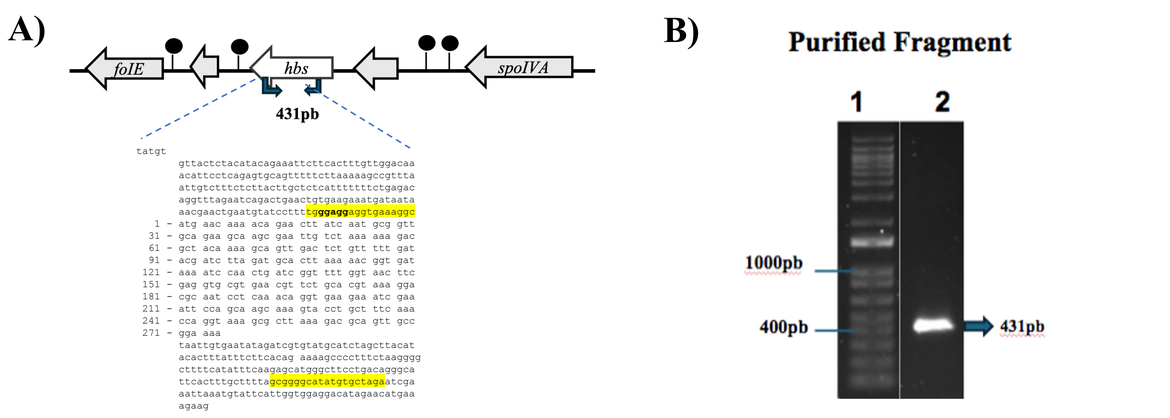

Fig. 2. PCR to amplify the open reading frame of the *Bacillus subtilis hbs* gene. **A)** Schematic representation of the gene of interest that will be amplified. **B)** PCR amplification. The amplicon generated is 431bp. **(1)** 1kb molecular size markers. **(2)** *hbs* gene amplified by PCR

Construction of a pJET1.2/blunt-*hbs* plasmid and its confirmation by restriction analysis

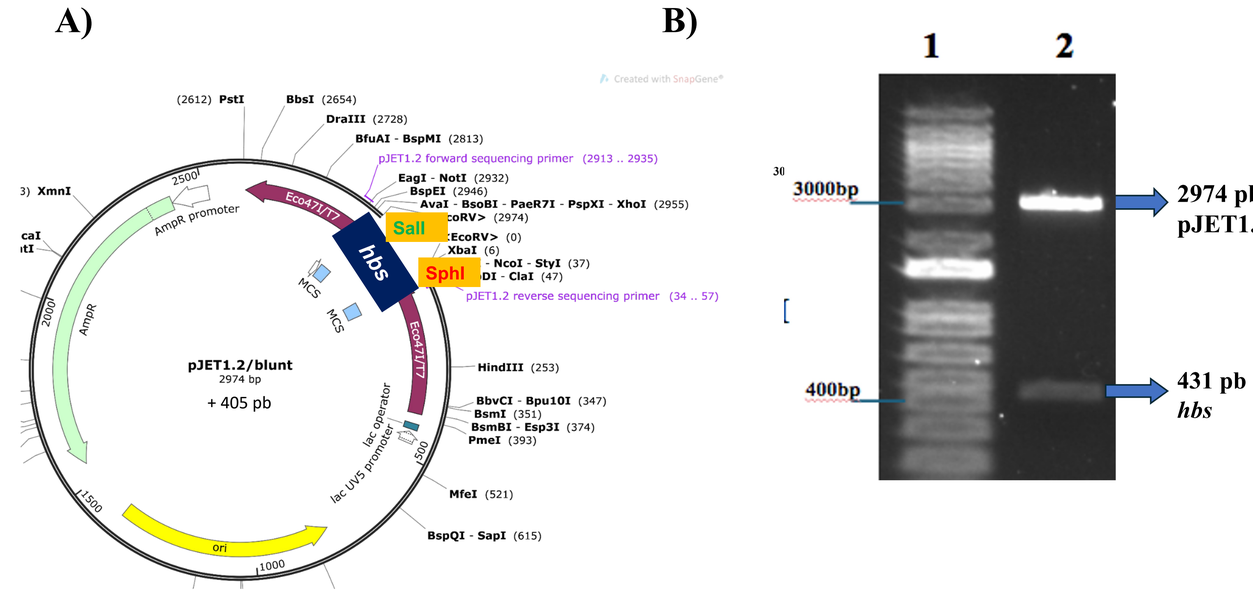

Fig. 3. pJET1.2/blunt-*hbs*construct. **A)** Schematic representation of the construction with the maintenance vector pJET1.2/blunt plus the open reading frame of the *hbs* gene. **B)** Corroboration of the pJET1.2/blunt-*hbs* construct by restriction with SaII and ShpI enzymes. **(1)** Molecular size markers 1kb **(2)** Restriction using SaII and SphI enzymes and obtention of the two fragments.

Obtention of a expression pDR111-*hbs* construct and its confirmation by restriction analysis

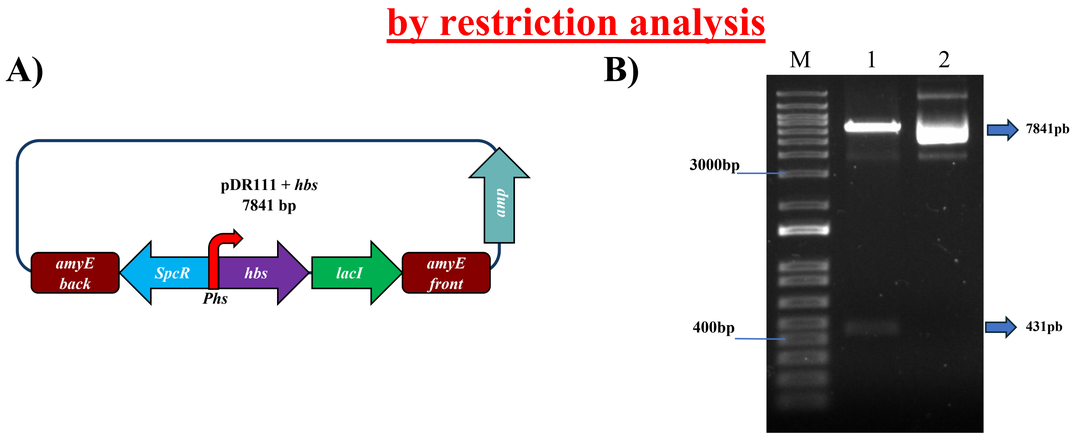

Fig. 4. Generation and characterization of a pDR111-*hbs* construct. **A)** Schematic representation of the construction with the pDR111 expression vector plus the hbs ORF. **B)** Obtaining strains overexpressing the hbs gene in *E. coli* and corroborating the overexpression by means of enzymatic restriction **(M)** 1 kb molecular size markers, **(1)** Restriction of the plasmid using the enzymes SaII and SphI. It can be observed the obtaining of a band of 7841 bp that corresponds to plasmid pDR111 and a band of 431 bp that corresponds to the hbs fragment **(2)** Uncut plasmid of clone 12.

Genetic transformation

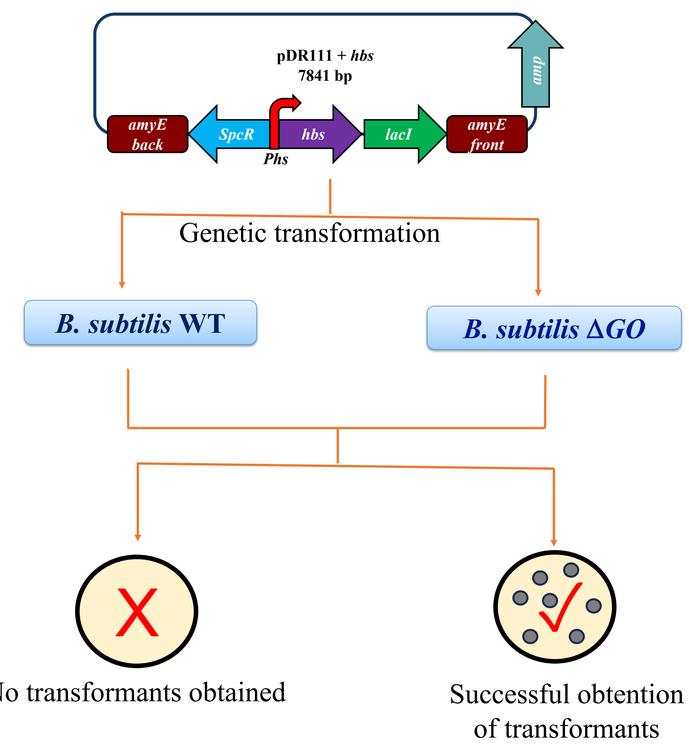

Fig. 5. Genetic tansformation with the P*hs-hbs* construct only generated viable colonies in the GO-deficient but no in the WT strain.

Conclusions

1) A genetic construct to overexpress *hbs* was generated
2) The Phs-hbs construct was sucessfully transformed into the GO-deficient but not in the suggesting that the gene *hbs* contributes to the hyper resistance to oxidative stress.

References

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