Influence of Sigma B-mediated General Stress Response in resistance and germination of *Bacillus subtilis* Spores

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INTRODUCTION

Sporulation constitutes one of the best adaptative strategies that allows spore-forming bacteria to survive under starvation and a wide range of environmental stresses. It is well known that variations in sporulation conditions from the optimal, such as temperature, influence spore resistance to physical and chemical treatments and germination dynamics, although several aspects of the mechanism and regulation of these changes remain unknown[1]. In vegetative cells, Sigma B (SigB)–mediated general stress response has a pivotal role in stress resistance and growth in adverse environments[2], but very little is known about the role of SigB response in resistance and germination in *B. subtilis* spores.

OBJECTIVE

Study of the influence of **SigB** – mediated stress response on **resistance** and **germination** of ***B. subtilis*** **spores**

MATERIALS AND METHODS

The strains of *B. subtilis* 168 used in this work were: wild type (WT), *sigB::erm* (lacking the SigB subunit of RNA polymerase[2]), *rsbX::erm* (lacking a SigB-repressor, RsbX[2]) and *rsbS::erm* (lacking a SigB-repressor, RsbS[2]).

HEAT INACTIVATION KINETICS

Spores were treated at 105.0°C, 102.5°C, 100.0°C and 97.5°C in a thermoresistometer TR-SC. Inactivation curves were fit to the Log-linear + shoulder model (Geeraerd et al., 2000)[3] and D values were calculated as $Sl + 2.303/Kmax$.

CHEMICAL INACTIVATION

Spores were treated with:

- Dodecylamine (1 mM) at 45°C for 3 h.
- Sodium hypochlorite (2.5 g/L) at 25°C for 30 min.

GERMINATION KINETICS

Germination was triggered with L-alanine (L-Ala), L-valine (L-Val) and AGFK (arginine, D-glucose, D-fructose and potassium) at 37°C and calcium dipicolinic acid (CaDPA) at 30°C. Germination curves were fit to the exponential decay model.

RESULTS AND DISCUSSION

HEAT INACTIVATION KINETICS

Figure 1 shows inactivation curves at 100.0°C and Table 2 the corresponding $D_{100.0°C}$ and z values. The absence of SigB did not affect spore heat resistance, whereas mutants *rsbX::erm* and *rsbS::erm* were significantly more sensitive than the WT strain but have a similar z value.

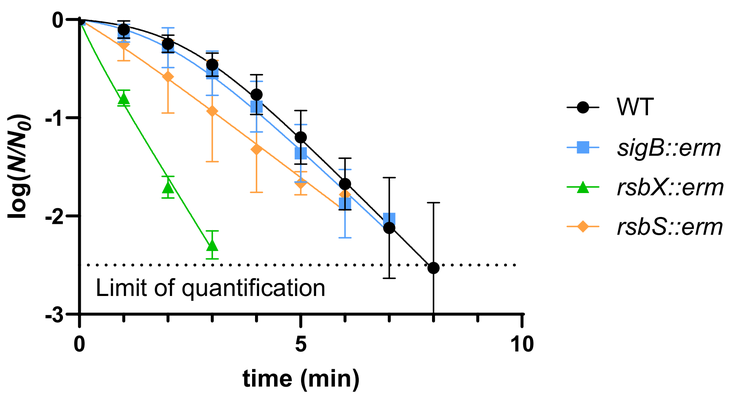

Figure 1: Inactivation curves at 100.0°C of spores from the indicated strains. Error bars show the standard deviation of means.

	$D_{100°C}$ (min)	z (°C)
WT	4.27 (0.55)	6.82 (0.57)
sigB::erm	4.31 (0.71)	7.02 (0.31)
rsbX::erm	1.48 (0.27)**	7.37 (0.72)
rsbS::erm	3.23 (0.98)*	6.35 (0.13)

Table 2: Means and standard deviations of $D_{100°C}$ and z values of the indicated strains. Statistically significant differences between each mutant and the WT strain are pointed with asterisks (*$P \leq 0.05$, **$P \leq 0.01$).

CHEMICAL INACTIVATION

We also tested the resistance of the *sigB::erm* and *rsbX::erm* mutant to dodecylamine and sodium hypochlorite. The absence of SigB or RsbX increased resistance to dodecyalmine in comparison to the WT strain (Fig. 2A). On the contrary, *rsbX::erm* spores were slightly more sensitive to sodium hypochlorite than WT and *sigB::erm* spores (Fig. 2B).

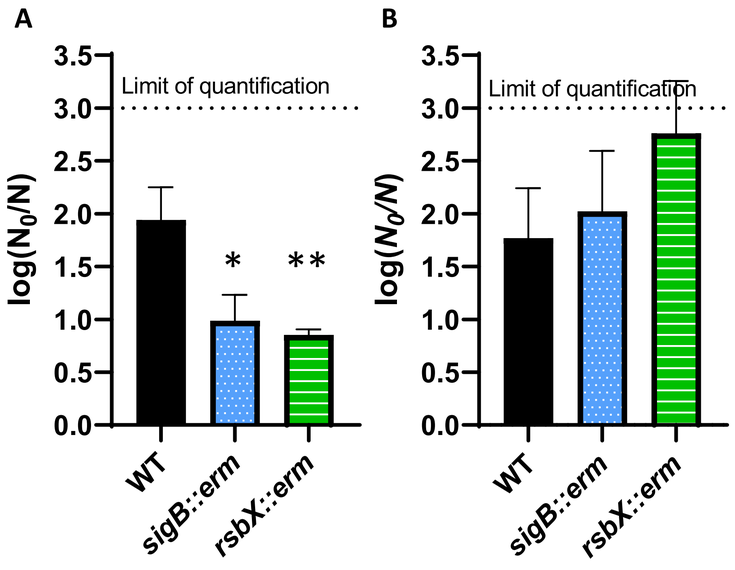

Figure 2: Inactivation of spores from the indicated strains by dodecylamine (1 mM; **A**) or sodium hypochlorite (2.5 g/L; **B**). Error bars represent the standard deviation of means and asterisks indicate the statistically significant differences with the WT strain (*$P \leq 0.05$, **$P \leq 0.01$).

GERMINATION KINETICS

Germination rate of the *rsbX::erm* mutant was significantly lower than that of the WT, *sigB::erm* and *rsbS::erm* strain against L-Ala (Fig. 3A), L-Val (Fig. 3B) and CaDPA (Fig. 3C). However, when exposed to AGFK, the *sigB::erm* mutant germinated slower than the WT and *rsbX::erm* strain, while the *rsbS::erm* mutant showed higher germination rate (Fig. 3D).

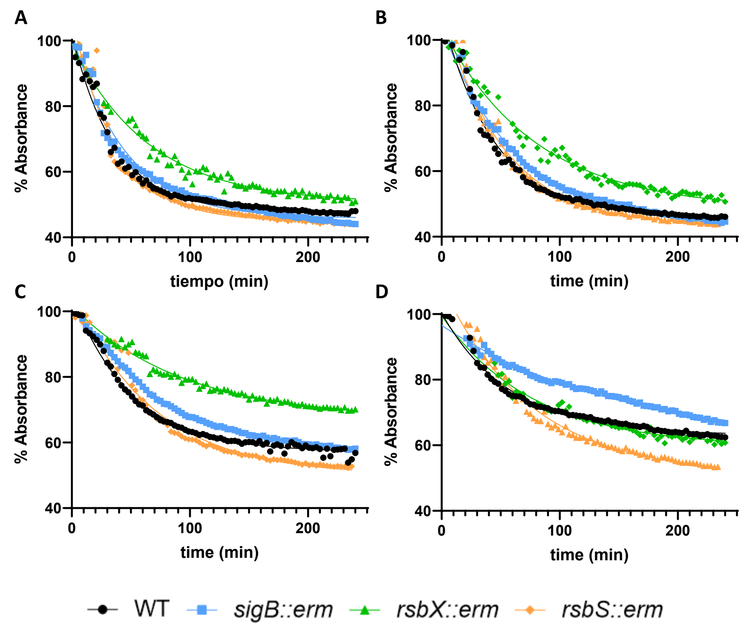

Figure 3: Germination kinetics of the indicated strains in the presence of L-Ala (**A**), L-Val (**B**), CaDPA (**C**) and AGFK (**D**). Data points represent the mean values of three replicates, and error bars have been omitted for clarity.

CONCLUSIONS

Unlike in vegetative cells, increased levels of SigB by lacking the repressor RsbX largely diminishes spore heat resistance at any treatment temperature.

Both downregulation and upregulation of SigB activity confers spore resistance to dodecyalmine.

Increased levels of SigB through the absence of RsbX impaired germination rate in response to L-Ala, L-Val and CaDPA, but not to AGFK.

REFERENCES

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3. Geeraerd et al. (2000). *International Journal of Food Microbiology. 59(3):185-209*

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