Effect of sublethal shocks on *Staphylococcus aureus* envelope properties: relationship with the development of cross-resistance to Pulsed Electric Fields

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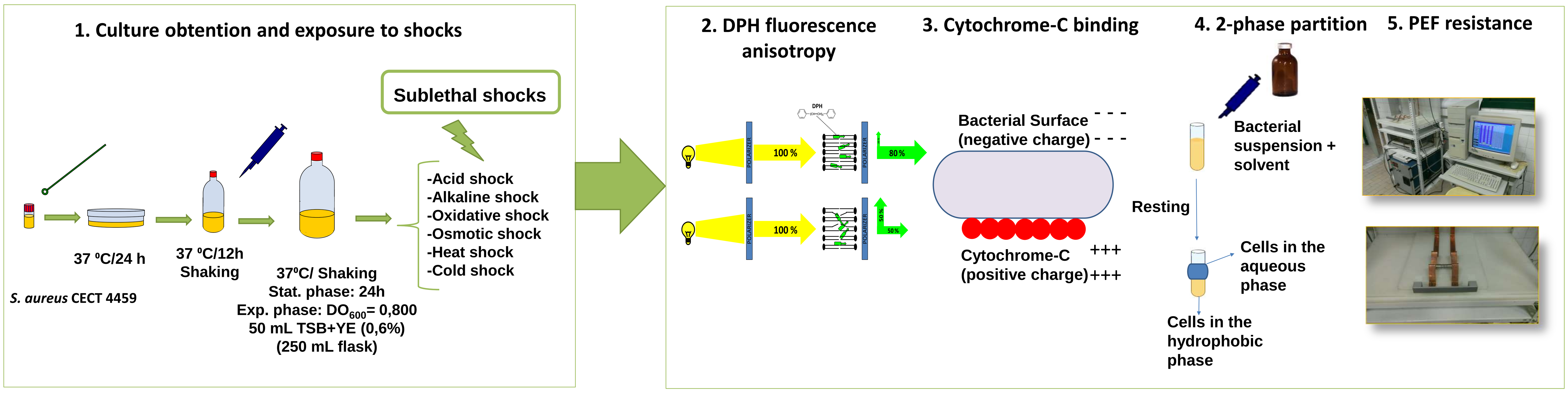
Introduction/objectives/aims

Heat is a technology widely used in the food industry for inactivating pathogenic and spoilage microorganisms. Nevertheless, it causes some undesirable effects on foods, leading to quality losses. Therefore, the food industry is interested in exploring different alternatives to heat for microbial inactivation [1]. Pulsed Electric Fields (PEF) is one of the most promising alternatives to heat treatments for food preservation but much research effort is still required to fully elucidate the factors affecting microbial inactivation by this technology, including the potential development of cross-resistance responses towards it [2]. The aim of this work was to study the effect of sublethal shocks of different nature on the properties of *Staphylococcus aureus* envelopes and its relationship with the development of cross-resistance to PEF.

Methods



Results

As can be observed in Figure 1, results obtained indicate that only heat shock resulted in a significant change ($p<0.05$) of *S. aureus* membrane fluidity (*r* value, which is inversely correlated to membrane fluidity, increased up to 0.05 units). This effect was more pronounced in exponential growth phase cells.

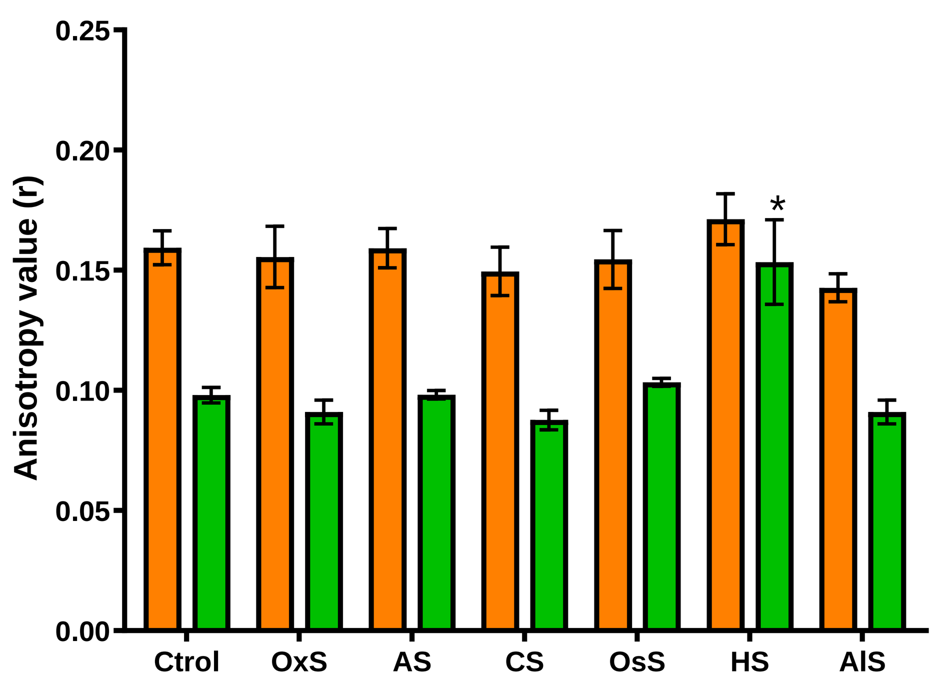


Figure 1. *r* values calculated for *S. aureus* CECT 4459 cells in stationary (■) and exponential (■) phase of growth. Asterisks (*) indicate values statistically different from control values (t-test p=0.05).

Heat shock also resulted in an increase (10.6 %) in surface hydrophobicity of stationary phase cells. Acid shock caused just the opposite effect, especially in the case of exponential phase cells, as it led to a decrease in surface hydrophobicity (Figure 2A).

Heat and alkaline shocks resulted in an increase in the surface negative charge of *S. aureus* cells, which was evidenced with a higher absorbance decrease of cytochrome C (up to 23 %).

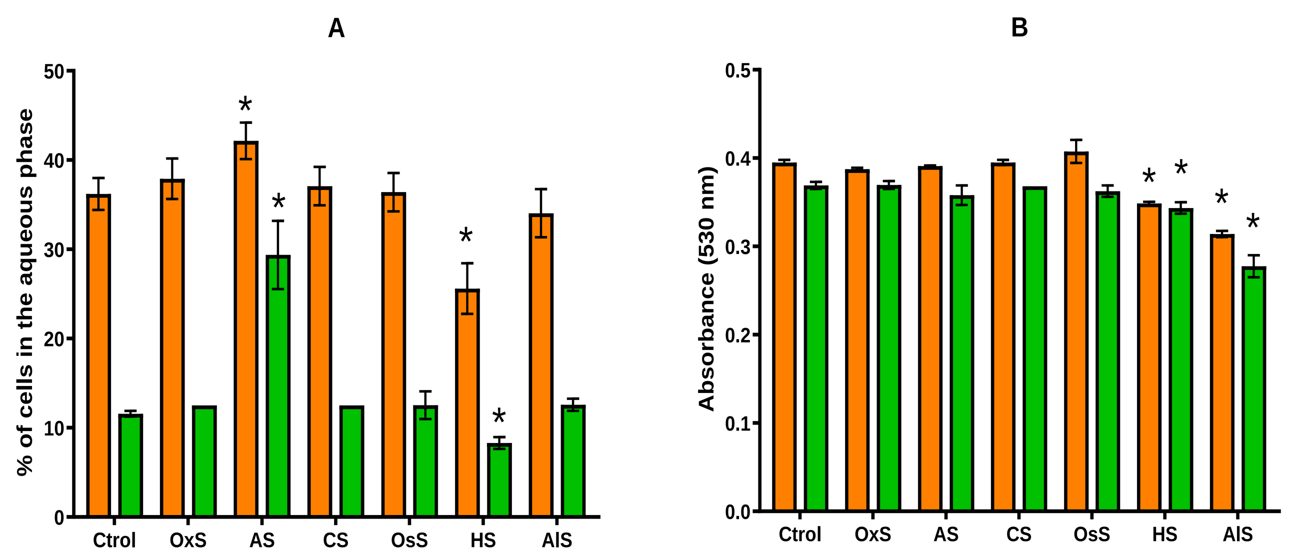


Figure 2. A) % of *S. aureus* CECT 4459 cells in the aqueous phase. B) Absorbance (530 nm) of the supernatant after incubation with the cytochrome. Cells in stationary (■) and exponential (■) phase of growth. Asterisks (*) indicate values statistically different from control values (t-test p=0.05).

On the other hand, heat and alkaline shocks were the only ones triggering an increase in PEF resistance (3- and 6-fold increase in the time for the first decimal reduction) [2]. Neither the changes in surface charge nor the heat and alkaline shock-dependent development of PEF resistance required *de novo* protein synthesis (Table 1).

Adpatation medium	Control cells C	Control cells S.D.	Alkaline Shock C	Alkaline Shock S.D.	Heat Shock C	Heat Shock S.D.
Stationary growth phase						
W/I	2.29	0.21	0.90	0.03	1.51	0.17
Chloramphenicol	-	-	0.90	0.04	1.30	0.19
Rifampicin	-	-	0.90	0.03	-	-
Cerulenin	-	-	0.96	0.08	1.85	0.02
Exponential growth phase						
W/I	3.57	0.42	1.97	0.44	2.46	0.14
Chloramphenicol	-	-	2.04	0.34	2.27	0.19
Rifampicin	-	-	2.15	0.21	2.81	0.25
Cerulenin	-	-	1.95	0.01	2.51	0.07

C: Log cycles of inactivation after 50 pulses (26 kV/cm).

W/I: without inhibitors.

Table 1. Log cycles of inactivation (C) and standard deviations (S.D.) after 50 pulses (26 kV/cm) of stationary and exponential growth phase cells of *S. aureus* CECT 4459 before (Control) and after a 120 minutes alkaline (pH 9.5) shock and heat (45ºC) shock without (W/I) or with the addition of inhibitors.

Conclusions

❖ Sublethal shocks leading to an increase in *S. aureus* PEF resistance also led to changes in the surface charge of *S. aureus* cells, thus suggesting that surface charge would play a major role in *S. aureus* PEF resistance.

References

[1] Mañas, P., & Pagán, R. (2005). Microbial inactivation by new technologies of food preservation. *Journal of Applied Microbiology*, 98, 1387e1399.
[2] Cebrián, G., Raso, J., Condón, S., & Mañas (2012). Acquisition of pulsed electric fields resistance in *Staphylococcus aureus* after exposure to heat and alkaline shocks. *Food Control*, 25, 407-414.

Acknowledgements

Laura Nadal gratefully acknowledges the financial support for her studies provided by the "Ministerio de Educación y Formación Profesional" (FPU18/04898).