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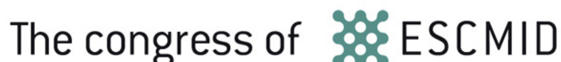

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Generation and characterization of persister cells of Staphylococcus aureus and Pseudomonas aeruginosa

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Background: Persister cells are a small subpopulation of non-replicating, metabolically quiescent bacteria that exhibit multidrug tolerance to antibiotics. They are largely responsible for the recalcitrance of most chronic infections (e.g. biofilm-associated infections) to the antimicrobial treatment, thus representing a great challenge for therapy. Unfortunately, the low frequency of persister cells within bacterial cultures constitutes a significant obstacle for the study of persistence and the development of novel antimicrobial drugs able to target persisters. The present study examined the possibility to obtain bacterial populations with high levels of persisters through chemical treatment. In particular, we investigated the ability of the uncoupling agent carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) to induce a persister status in cultures of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two bacterial species commonly involved in recurrent and relapsing infections.

Material/methods: Stationary-phase cultures of *S. aureus ATCC 33591* and *P. aeruginosa ATCC 27853* were exposed to CCCP for 3 h to induce persistence. The metabolic profile of CCCP-treated bacteria was fully characterized by monitoring bacterial heat production through isothermal microcalorimetry and by flow cytometry after staining with a fluorescent indicator of bacterial oxidoreductase activity. CFU counting was performed in parallel to analyze the effect of CCCP on cell viability. In order to verify the successful induction of the persistent phenotype, CCCP-pretreated cells were exposed to high concentrations (10xMIC) of antibiotics and their viability was assessed by CFU counting. Finally, the time required for the revival of induced persisters and their susceptibility to antibiotics after the removal of CCCP was determined by measuring in real-time heat production.

Results: Exposure of *S. aureus* and *P. aeruginosa* cultures to optimized concentrations of CCCP determined a global reduction of the bacterial metabolic activity with only a minor effect on cell viability. Indeed, CCCP-treated cells were characterized by a substantial decrease in heat production as compared to the untreated control. They also displayed a low reductase activity as proven by the reduction in fluorescence signals after treatment. Furthermore, CCCP-pretreated bacteria exhibited an antibiotic-tolerant phenotype as they resulted highly insensitive to different classes of antibiotics. After CCCP removal, induced persisters showed a delay in heat production that could correlate with the presence of a lag phase before resumption of normal growth. The metabolic reactivation of bacteria coincided with their reversion to an antibiotic-sensitive phenotype as stated by the drop in heat production following exposure to different antibiotics.

Conclusions: CCCP treatment represents a suitable method to induce persistence at high efficiency in medically relevant Gram-positive and Gram-negative bacterial species. Reduction in metabolism, high antibiotic-tolerance and reversion to a normal-growing, antibiotic-sensitive phenotype after stress removal confirmed the development of the persister status in CCCP-treated bacterial populations.