

## Medium supplementation with magnesium prevented the induction of dormancy in biofilms of clinical and commensal isolates of *Staphylococcus epidermidis*

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*Staphylococcus epidermidis* is a commensal bacterium that colonizes the skin and mucous membranes, being the most prevalent staphylococcal species found in humans. However, *S. epidermidis* has the ability of colonize indwelling medical devices establishing biofilms, which makes this bacteria a common cause of bacteraemia particularly in immunocompromised individuals and neonates [1][2]. The presence of high amounts of dormant bacteria (viable but non-culturable cells, VBNC) is a hallmark of biofilms, making them more tolerant to antimicrobials and elusive to the host immune response [3]. Glucose (G) is commonly used *in vitro* to induce a biofilm mode of growth in *S. epidermidis* cultures [4] due to the medium acidification derived from carbon metabolism [5]. Previous studies in our laboratory demonstrated that the accumulation of VBNC bacteria inside *S. epidermidis* biofilms could be prevented by supplementation of the medium with magnesium (Mg) (unpublished data). In the present work, a flow cytometric live/dead staining [6], in combination with the spread-plate method, were used to evaluate the effect of magnesium in preventing dormancy on glucose excess-grown biofilms in 53 strains of international clinical, Portuguese clinical, and Portuguese commensal isolates of *S. epidermidis*. Culture supplementation with Mg<sup>2+</sup> prevented the accumulation of death bacteria inside the biofilms in all the groups of strains tested, being the proportion of live bacteria (SYBR<sup>®</sup>PI) significantly increased by Portuguese clinical isolates. The quantification of colony forming units (CFUs) revealed that Mg hampered the induction of dormancy in all the groups of strains studied (International clinical G:  $3,6 \pm 0,8 \times 10^7$  vs. G+Mg:  $2,6 \pm 0,5 \times 10^8$ , Portuguese clinical  $6,8 \pm 2,0 \times 10^7$  vs.  $4,3 \pm 1,4 \times 10^8$ , Portuguese commensal  $7,2 \pm 1,5 \times 10^7$  vs.  $2,6 \pm 0,4 \times 10^8$  CFUs/mL). These results confirmed that dormancy modulation is widespread in different *S. epidermidis* isolates obtained from clinical and commensal sources, suggesting that this is indeed an important mechanism of *S. epidermidis* biofilm physiology.

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