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Spheroplasts preparation boosts the catalytic potential of a squalene-hopene cyclase

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PURPOSE OF THE ABSTRACT

Squalene-hopene cyclases (SHCs) are a highly attractive class of membrane-bound enzymes to synthesize terpenes which are then used in the fragrance industry and as bioactive molecules [1-3]. Typically, SHCs are used as whole-cell biocatalysts, but they suffer from the outer cell membrane acting as a diffusion barrier for the highly hydrophobic substrate/product. Alternatively, SHCs have been used as purified enzymes but their stability is very poor [1]. In this work, we present the application of SHC spheroplasts [4]. This unexplored strategy for biocatalysis consist of the (partial or total) removal of the outer cell membrane of E. coli cells used as biocatalysts. SHC spheroplasts have improved the enzymatic cyclization of a set of terpenes (squalene, geranyl acetone, farnesol, and farnesyl acetone) up to 100-fold (Figure 1). In addition, we introduce a new concept for the carrier-free immobilization of spheroplasts via crosslinking, which is called CLS (crosslinked spheroplasts). CLS offered the advantage of biocatalyst reusability while maintaining the same activity as the spheroplasts. To sum up, SHC spheroplasts are an innovative approach to overcome the notoriously challenging stereoselective head-to-tail cyclization of terpenes in an straightforward and sustainable manner.

FIGURES

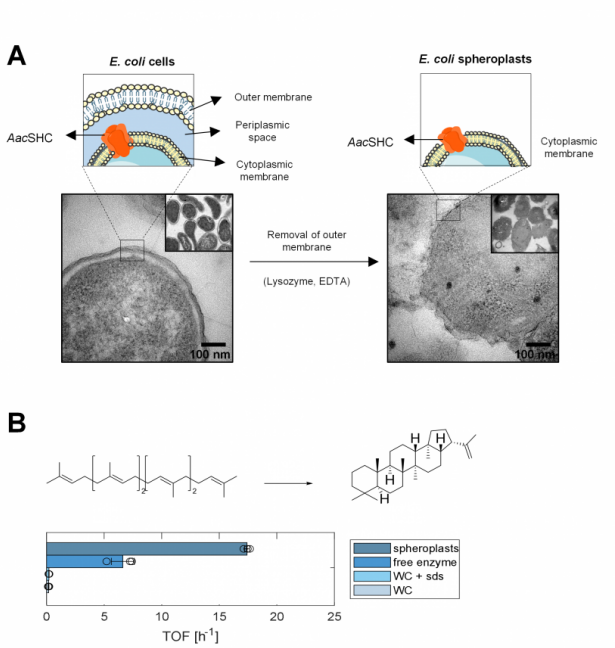

FIGURE 1

SHC spheroplasts compared to WC (whole cells) biocatalysts.

A) Scheme (on the top) and TEM images (at the bottom) of SHC spheroplasts and whole cells. B) SHC-mediated cyclization of squalene by spheroplasts, purified enzyme, WC with SDS and WC. TOF: turnover frequency per hour.

FIGURE 2

KEYWORDS

Squalene-hopene cyclase | enzyme immobilization | Spheroplasts | terpene cyclazation

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