Mycobacterium smegmatis is a suitable cell factory for the production of steroidal synthons - DTU Orbit (18/02/2019)

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A number of pharmaceutical steroidal synthons are currently produced through the microbial side-chain cleavage of natural sterols as an alternative to multi-step chemical synthesis. Industrially, these synthons have been usually produced through fermentative processes using environmental isolated microorganisms or their conventional mutants. Mycobacterium smegmatis mc2155 is a model organism for tuberculosis studies which uses cholesterol as the sole carbon and energy source for growth, as other mycobacterial strains. Nevertheless, this property has not been exploited for the industrial production of steroidal synthons. Taking advantage of our knowledge on the cholesterol degradation pathway of M. smegmatis mc2155 we have demonstrated that the MSMEG_6039 (kshB1) and MSMEG_5941 (kstD1) genes encoding a reductase component of the 3-ketosteroid 9α-hydroxylase (KshAB) and a ketosteroid Δ1-dehydrogenase (KstD), respectively, are indispensable enzymes for the central metabolism of cholesterol. Therefore, we have constructed a MSMEG_6039 (kshB1) gene deletion mutant of M. smegmatis MS6039 that transforms efficiently natural sterols (e.g. cholesterol and phytosterols) into 1,4-androstadiene-3,17-dione. In addition, we have demonstrated that a double deletion mutant M. smegmatis MS6039-5941 [ΔMSMEG_6039 (ΔkshB1) and ΔMSMEG_5941 (ΔkstD1)] transforms natural sterols into 4-androstene-3,17-dione with high yields. These findings suggest that the catabolism of cholesterol in M. smegmatis mc2155 is easy to handle and equally efficient for sterol transformation than other industrial strains, paving the way for valuating this strain as a suitable industrial cell factory to develop à la carte metabolic engineering strategies for the industrial production of pharmaceutical steroids.