重組 *Photobacterium damselae* 半胱胺酸-S-共軛 β-裂解酶 (MetC) 釋放葡萄酒含硫香氣化合物 4-巰基-4-甲基-2-戊酮和 3-巰基-1-己醇之潛力

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摘要

葡萄酒中常見主要揮發性芳香硫醇為 4-巰基-4-甲基-2-戊酮 (4-Mercapto-4-methyl-2-pentanone, 4MMP) 和 3-巰基-1-己醇 (3-Mercapto-1-hexanol, 3MH)，帶有黃楊木、黑加侖、百香果等風味，因其具有極低之感官閾值，於氣味中作為重要貢獻者。由於市面上多為化學合成之香料，消費者對天然香料的需求逐漸上升，因此本篇擬以 *Photobacterium damselae* subsp. *damselae* 之重組蛋白 MetC 作為對象，研究酵素從前驅物 S-3-(hexan-1-ol)-l-cysteine (Cys-3MH) 和 S-4-(4-methylpentan-2-one)-l-cysteine (Cys-4MMP) 釋放 3MH、4MMP 之潛力。首先將合成之前驅物 Cys-3MH 透過超極致液相層析串聯質譜儀 (Ultra performance liquid chromatography-Tandem mass spectrometry, UPLC-MS/MS) 鑑定，確認其為目標前驅物。以含有 *metC* 基因的載體 pET-21a 之 *Escherichia coli* C43 (DE3) 純化出 MetC，再以電白質電泳 (44 kDa)、胜肽鑑定 (Protein ID) 確認其身分為半胱胺酸-S-共軛 β-裂解酶 (Cysteine-S-conjugate β-lyase) 並定量。接著評估活性，得到 MetC 之最適反應條件為含有磷酸吡哆醛 (Pyridoxal 5'-phosphate, PLP) 輔因子下於 30°C、pH 9 作用，動力學結果顯示 MetC 對 L-cystathionine 的裂解速率較高 (V_{max} = 10.63 和 18.02 μM/min)，對 L-cystine 的親和力較高 (K_m = 0.09 mM)，並於有輔因子 PLP 之狀況下酵素催化能力較佳 (k_{cat}/K_m = 9.3×10^3 和 9.5×10^3 M^{-1}s^{-1})。綜合上述，*Ph. damselae* subsp. *damselae* 之重組蛋白 MetC 為半胱胺酸-S-共軛 β-裂解酶，具有作為前驅物 Cys-3MH、Cys4MMP 轉化香氣化合物 3MH、4MMP 之酵素潛力。

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