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Attempts to identify ecm38 suppressor in the yeast strain yph499 responsible for red pigmentation

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Abstract:

Knockouts of the ADE2/ADE1 in Saccharomyces cerevisiae gene results in the accumulation of the toxic intermediates AIR/CAIR. The detoxification of these intermediates occurs through conjugation with GSH using GRX4. On transport of the GSH conjugated intermediates to vacuole, ECM38, a transpeptidase, processes the conjugated adducts further and generates glutamine and cys-gly dipeptide leading to red pigmentation in the vacuole. The knockout of ECM38 results in loss of red pigmentation in BY4742 strain. Surprisingly, another yeast strain YPH499 congenic to S288C shows red pigmentation even after ECM38 disruption. This indicates the presence of a different gene compensating for the ECM38 activity. In this study, we used varying approaches to identify the suppressor gene present in YPH499 strain. A SNP in the CCC1 gene was discovered during sequence analysis of the BY4742 and YPH499 vacuolar genes, and when further investigated for a putative role in pigmentation, no involvement in pigmentation was found. In continuation of our conjecture as an indirect approach, we attempted to generate a genomic library of YPH499 strain. In the same light, random mutagenesis using EMS was also utilized to obtain the desired mutant. Since Ecm38 is a vacuolar transpeptidase, we postulated that one of the other vacuolar peptidases could function as a suppressor gene. Currently, we are examining the role of the vacuolar carboxypeptidases and aminopeptidases in pigmentation through a disruption approach.

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