Molecular characterisation of *Lipomyces kononenkoae* α-amylase LKA1.

[1]**Ramachandran, N.**, [1,2]Pretorius, I.S. and [1]Cordero Otero, R.R.

[1]Institute for Wine Biotechnology, Stellenbosch University, Stellenbosch, South Africa; [2]Australian Wine Research Institute , Adelaide, Australia.

The α-amylase from *Lipomyces kononenkoae* LKA1 is active on α-1,4 and α-1,6 linkages in starch and related substrates. In addition to raw starch degradation, the enzyme exhibits considerable activity on pullulan. The primary structure of LKA1 exhibits clear sequence similarities and a predicted common super secondary fold $(\alpha/\beta)_8$ barrel structure, as well as a C-terminal stability domain shared with other members of the α-amylase family. The catalytic domain of α-amylases consists of four conserved segments that constitute the active domain and are well conserved in LKA1. However, the invariant His296 of the fourth conserved segment (TAKA amylase numbering), which has been highly conserved and well demonstrated in transition-state stabilisation in other α-amylases, is replaced by Gln443 in LKA1. Additionally, the N-terminal region of LKA1 preceding the catalytic domain consists of 132 amino acids shows 36% identity to the only known N-terminal starch-binding domain of *Rhizopus oryzae* glucoamylase. Mutant enzymes of LKA1 were constructed to evaluate the significance of the unique N-terminal region and the role of the variant Gln443 at the catalytic region. LKA1δ N lacking 132 amino acids at the N-terminal region shows a 95% reduction in raw starch substrate adsorption, 20% less hydrolytic activity in complex starches and 30% less thermal stability than the wild-type enzyme. Site-specific mutagenesis of Gln443 has confirmed the essential role of this amino acid in the substrate specificity of LKA1. The effect of mutations on the pH and temperature profile of the enzyme, and specificity towards substrates, will be discussed.