THE SENSITIVITY OF YEASTS TO KILLER YEAST TOXINS:
WITH FOCUS ON THE
KILLER YEAST PICHIA MEMBRANIFACIENS

by

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ABSTRACT

The yeast killer phenotype is defined by a yeast's ability to secrete a toxin that is lethal to other yeast strains, but to which they are themselves immune. An investigation was undertaken to identify a yeast with broad spectrum killer activity towards indigenous non-\textit{Saccharomyces} yeasts of the wine ferment. The growth of these indigenous yeasts during wine fermentation may result in inappropriate sensory properties to the wine.

The sensitivity of tester strains characteristic of the wine ferment microflora to 14 killer yeasts were assayed at pH 4.5, revealing a total of 147 killer-sensitive reactions. At a pH comparable to a wine ferment (pH 3.5), only 28\% of these 147 killer-sensitive reactions were observed. Intraspecific differences in killer susceptibility were identified for strains of \textit{Pichia anomala}, \textit{Kluyveromyces lactis} (two strains) and \textit{Pichia membranifaciens}.

To gain further insight into the killer phenotype of \textit{Pichia membranifaciens}, the killer activity of ten \textit{Pichia membranifaciens} strains was assayed towards 15 tester strains. Intron primer PCR confirmed the ten \textit{Pichia membranifaciens} strains to be related, but different to, the type strain of \textit{Pichia membranifaciens}. Based on their killer activity each \textit{Pichia membranifaciens} strain was allocated one of four possible killer types.

The killer phenotype of the two strains of the Class C killer type, \textit{Pichia membranifaciens} CBS 638 and the type strain CBS 107, was found to be encoded by nuclear genes. In contrast, the killer strains of the Class B and D killer types harboured an extrachromosomal element of the same molecular weight. For \textit{Pichia membranifaciens} CBS 7374 of Class D this extrachromosomal element (pPM01) was determined to be a dsRNA in nature, however, its not known whether pPM01 is associated with the killer phenotype.

Of the ten \textit{Pichia membranifaciens} strains investigated, strain CBS 7374 displayed the broadest killing range. The \textit{Pichia membranifaciens} CBS 7374 killer toxin was found to be a heat liable protein with an acidic pI. Using a purification protocol developed in this study, a protein of 20.5 kDa was identified as a candidate for the \textit{Pichia membranifaciens} CBS 7374 killer toxin.

Investigating the sensitivity of tester yeasts to killer yeasts further revealed a petite of \textit{Saccharomyces cerevisiae} AWRI 1360 (p*K5), strain AWRI 1361 (p*K8), to be resistant to ten killer yeasts to which the parent was sensitive. This included resistance to the killer yeasts \textit{Saccharomyces cerevisiae} K2, \textit{Kluyveromyces lactis} var. \textit{lactis} and \textit{Willemiopsis ustus} var. \textit{murukii}, where the primary receptor and mode of action differs for each killer.
protein. This is the first known report of a mutant displaying resistance to more than one killer type. Characterisation of this petie revealed that its resistance to these killer toxins is attributed to a partially dominant, nuclear mutation. This mutation was found to be independent of oxidative-phosphorylation and yet, conferred resistance only in the presence of non-functional mitochondria. This study also revealed that for some strains, petites of sensitive parents showed a reduction in sensitivity to killer yeasts, and that this reduction in sensitivity was independent of oxidative-phosphorylation.
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