Talk

Identification and Characterization of Killer Yeasts

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ABSTRACT

Motivation: Killer factor is a virus like particle infecting Saccharomyces cerevisiae. Two lineal doubled stranded RNA episomes are necessary to maintain the killer phenotype, a defective M molecule encoding the killer toxin, and an L molecule encoding the capsid protein and polymerase essential for the maintenance of the M molecule. The killer factor is lethal to sensitive cells of the same or related yeast species, helping the producer strain to prevail in the yeast population. Studies have shown that killer yeasts are common and play an active role during grape must fermentation(1). Killer phenotype analysis is, therefore, essential to understand yeast population dynamics in wine production. So far, four killer viruses have been discovered (K1, K2, K28 and Klus), each one with a completely different toxin. In this context, an efficient and fast test to detect and classify killer yeasts is necessary. In this study, we are designing a PCR based protocol to detect killer yeasts and distinguish between K1, K2, K28 and Klus. We are using this test to study the presence and abundance of killer phenotype in flor yeasts, which grow when the fermentation is saturated.

Methods: We have designed a 3 pairs of primers to detect M1, M2 and Mlus, and 3 pairs of primers to detect L molecules (L1, L2 and Llus) upon retrotranscription of a nucleic acid preparation. These primers allow performing multiplex analysis. We use the traditional spot test method to confirm the results we obtain with RT-PCR test.

Results: Results prove that our test can distinguish successfully between K1, K2 and Klus. Moreover, the test can identify mixtures of killer yeasts in the same sample and allow us to check what kind of L molecule is associated with each M molecule. All results obtained have been confirmed successfully with the traditional spot test. Regarding flor yeasts, we have found that there is not a single killer yeast in 30 strains studied representing mayor flor yeast types present in Jerez and Montilla wine region. 24 strains are sensitive to K1 and K2, 7 are resistant to K2 and sensitive to K1 and 2 are resistant to both toxins.

Conclusions: We confirm that the RT-PCR test designed can identify and distinguish killer yeasts and mixed killer populations. The fact we are not able to find a killer yeast in a varied population of flor yeast may indicate that flor yeasts do not need killer factor to prevail or flor yeast genetic background is incompatible with killer factor.

REFERENCES

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