



## AP-14: *In vitro* antimycotic activity of a *Pichia kluyveri* killer protein against spoilage yeasts

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### Abstract

The control of spoilage yeasts in food and beverage manufacturing is managed mainly by using commercial chemical additives. However, some yeasts are resistant to many chemical preservatives. In this context, some recent studies proposed yeast killer proteins as novel compounds that could be used against food and beverage spoilage yeasts as an alternative to chemical preservatives. The present work aimed to purify and to characterize a novel killer toxin produced by a strain of *Pichia kluyveri* in order to study its *in vitro* activity against food and beverage spoilage yeasts and to check its efficacy in beverages.

In this study, the assay medium for checking killer activity was YEPG-MB agar (pH 4.5) using agar diffusion well bioassay method. The production of crude toxin by the killer strain was performed in YEPG broth medium (pH 4.0). The obtained toxin was applied to a gel filtration column and then analysed in SDS-PAGE. The minimum inhibitory concentrations MICs of the killer toxin was determined in flat-bottom 96-well microtiter plates by the microdilution method. Commercial pear juice and a soft drink were used for the evaluation of killer toxin activity and stability in beverages. Our results showed that the killer protein of *P. kluyveri* was active against food and beverage spoilage yeasts of the genera *Dekkera*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Torulasporea*, *Wickerhamomyces* and *Zygosaccharomyces*. After purification by gel filtration chromatography the killer toxin revealed a molecular mass of 54 kDa with SDS-PAGE. MICs of purified toxin exhibited a high *in vitro* activity against *D. bruxellensis* and *S. cerevisiae*. The killer toxin exhibited a dose–response effect against *D. bruxellensis* and *S. cerevisiae* in a soft drink and fruit juice, respectively.

In conclusion, we suggest that *P. kluyveri* killer toxin could be a novel food-grade compound for the control of food and beverage spoilage yeasts.

**Keywords:** *Pichia kluyveri*, Killer protein, Antimycotic activity, *In vitro* susceptibility, Spoilage yeasts.