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Antimicrobial activity of a peptides produced by *Saccharomyces cerevisiae, Wickerhamomyces anomalus* and *Tetrapisispora phaffii* against foodborne pathogens

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INTRODUCTION & AIM

In the food industry, synthetic preservatives are widely used to inhibit microbial spoilage, prevent nutritional and chemical changes, and extend shelf life [1]. However, growing consumer demand for healthier, minimally processed foods without synthetic additives has led to a shift toward natural preservation alternatives [2]. Pathogenic microorganisms like *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* spp. pose significant contamination risks to food products [3,4,5]. Various bioactive compounds with antimicrobial properties, including antimicrobial peptides, bacteriocins, and mycocins, have been identified as potential natural preservatives [6,7]. Therefore, this study aimed to assess the antimicrobial efficacy of peptide fractions from *Saccharomyces cerevisiae, Wickerhamomyces anomalus, and Tetrapisispora phaffii* against foodborne pathogens namely *E. coli* (ATCC 25922), *L. monocytogenes* (ISA 4008), and *Salmonella* spp. (ISA 4008). In addition, the shelf life of natural watermelon juice was tested in a 14-day challenge test.

METHOD

Alcoholic fermentations were performed in Yeast Extract Peptone Glucose medium (yeast extract 10 g/L; peptone 20g/L and 50 g/L of glucose) by *Wickerhamomyces anomalus , Tetrapisispora phaffii* and by *Saccharomyces cerevisiae* in a Synthetic grape must. The peptide fractions were obtained from the supernatant of the alcoholic fermentation carried out by each yeast Free-cell supernatants were filtrated using nitrocellulose membranes with 0.22 µm pores. The supernatant from the alcoholic fermentation of S. cerevisiae and W. anomalus was ultrafiltered using 10 kDa centrifugation units (Vivaspin 15R, Sartorius, Göttingen, Germany). (Vivaspin 15R, Sartorius, Göttingen, Germany) of 10 kDa and the permeate (<10 kDa) was concentrated 10-fold in a centrifugation unit equipped with a 2 kDa membrane. The T. phaffii fraction was obtained by centrifugation (Vivaspin 15R, Sartorius, Göttingen, Germany) at 10 kDa to obtain a fraction >10 kDa. The minimum inhibitory concentration (MIC) was determined using a microdilution assay in 96-well microplates. Peptide fractions incorporated into watermelon juice (pH 5.26) were analysed at 0, 1, 7 and 14 days in a challenge assay .

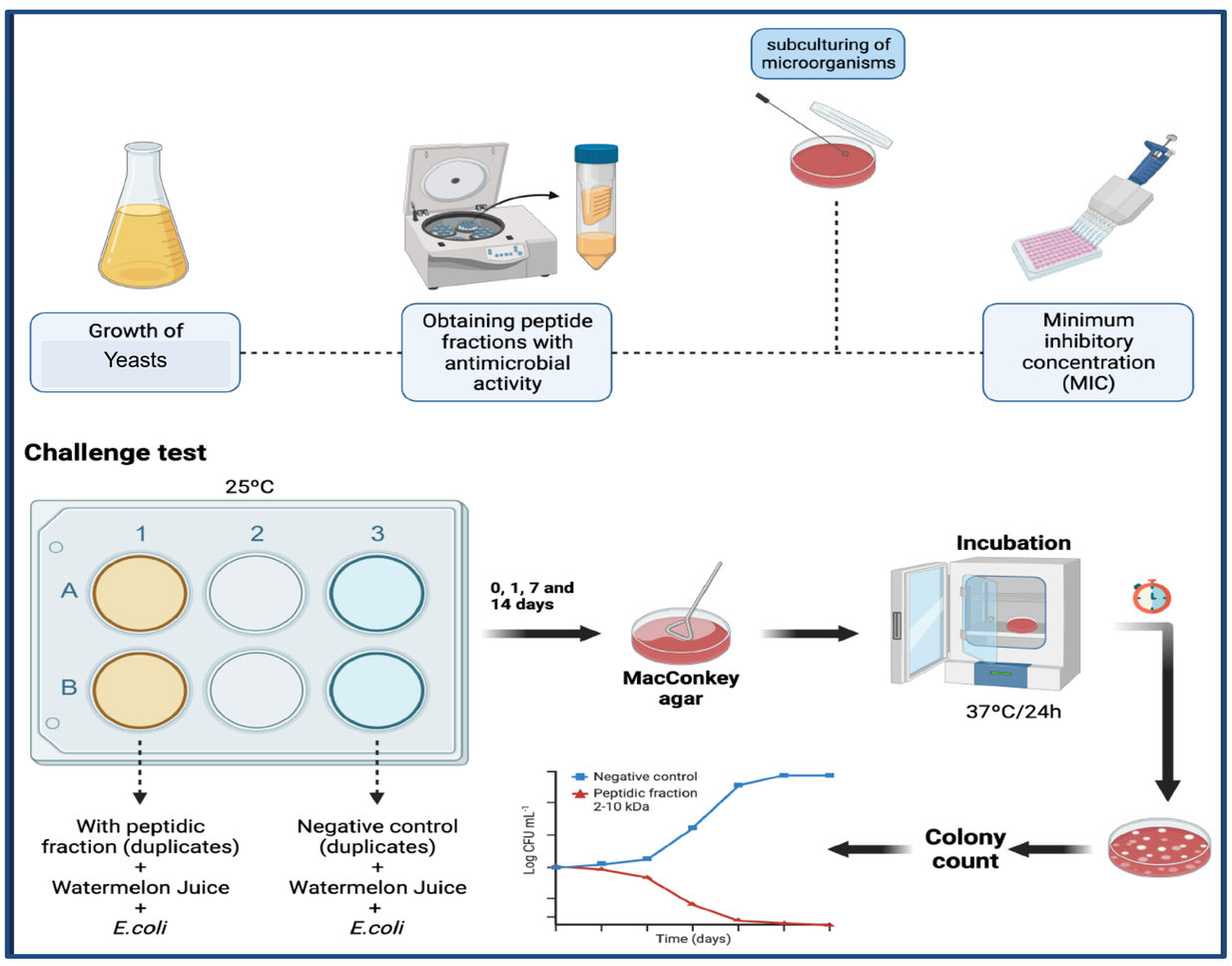

Figure 1: Diagram of the methodology

RESULTS & DISCUSSION

Minimum inhibitory concentration (MIC)

The peptide fraction from *S. cerevisiae* (2–10 kDa) exhibited the lowest MIC across all tested pathogens, indicating its superior antimicrobial potency (Table 1). Notably, all peptide fractions required higher concentrations to inhibit *L. monocytogenes* compared to *E. coli* and *Salmonella*, suggesting that *L. monocytogenes* is more resistant to these antimicrobial peptides.
While the peptide fractions from *W. anomalus* and *T. phaffii* demonstrated antimicrobial activity, they required higher MIC values than *S. cerevisiae*, indicating comparatively lower potency (Table 1)

Table 1 - MIC of the peptide fractions against the foodborne pathogens

Test strains	Minimum inhibitory concentration (MIC) (µg/mL)		
	W. anomalus (2-10 kDa)	*S. cerevisiae* (2-10 kDa)	*T. phaffii* (>10 kDa)
E. coli ATCC 25922	1150	225	1250
Salmonella sp. ISA 4348	1150	225	1250
L. monocytogenes ISA 4008	2300	450	2500

Challenge Test

Since the peptide fraction (2–10 kDa) derived from *S. cerevisiae* metabolism exhibited the strongest antimicrobial activity against *E. coli* (Table 1), it was selected for further testing in watermelon juice (Figure 2). Over a 7-day period, *E. coli* counts decreased from 6.28 log CFU/mL to below the detection limit (<1 CFU/mL), as determined by plate counting on MacConkey agar. No microbial growth was detected throughout the 14-day challenge test, indicating complete elimination of *E. coli*.

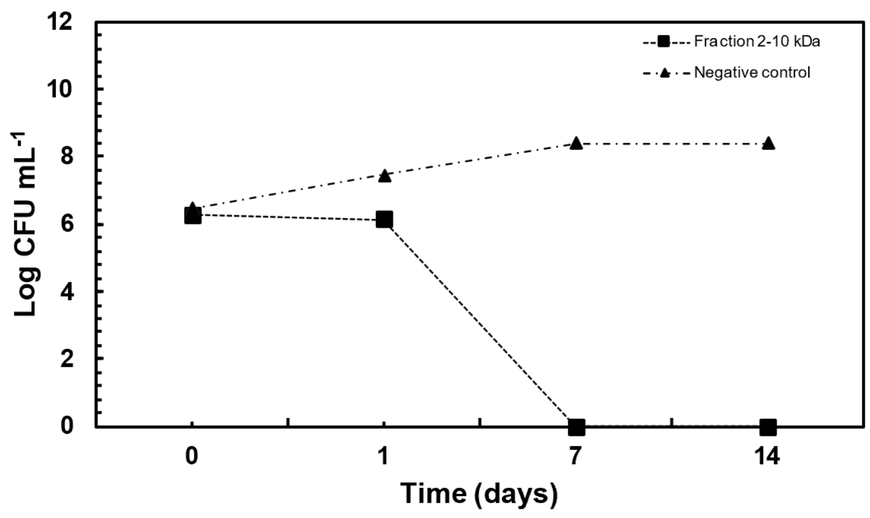

Figure 2 - Viability (Log CFU mL^{-1}) of *E. coli* over 14 days in the absence (negative control) and presence of the 2-10 kDa peptide fraction from the supernatant of an alcoholic fermentation carried out by *S. cerevisiae.*

CONCLUSION

The peptide fractions (2–10 kDa) derived from *W. anomalus* and *S. cerevisiae* metabolisms demonstrated antimicrobial activity against the tested foodborne pathogens, namely *E. coli, L. monocytogenes and Salmonella* spp. However, the results indicate that the peptide fraction from *S. cerevisiae* is the most effective antimicrobial agent, as it exhibited bactericidal activity against *E. coli* in watermelon juice. This highlights its potential as a promising candidate for food preservation and other antimicrobial applications.

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