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Antimicrobial and Antioxidant Peptides from *Wickerhamomyces anomalus*: A Natural Solution for Clean Label Food Preservation and Beyond

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INTRODUCTION & AIM

Foodborne diseases caused by microbial contamination are a global concern, resulting in adverse health effects and significant economic consequences such as increased public health expenses, food waste, and limitations on storage and transportation. Pathogenic microorganisms like *Escherichia coli, Listeria monocytogenes, Salmonella* spp., and *Candida* species pose a risk of contaminating food products [1-3]. To prevent the growth of these pathogens, preserve food quality, and extend shelf life, the food industry commonly uses chemical preservatives. However, many of these preservatives, such as sulphur dioxide, sodium benzoate, benzoic acid, sodium sorbate, and potassium sorbate, have potential risks to human health. As consumer interest in healthier foods without chemical preservatives increases, the food industry is under pressure to develop less processed and more natural products, known as "clean label" products. One emerging approach is the use of natural preservatives, such as bioactive metabolites produced by microorganisms. Numerous bioactive metabolites with antimicrobial activity, including antimicrobial peptides, bacteriocins, and mycocins, have been identified. *Wickerhamomyces anomalus* is a yeast with potential applications in the food industry due to its antimicrobial properties. This yeast can adapt to a wide range of conditions, including temperature and pH, making it versatile in different habitats. *W. anomalus* DBVPG 3003 produces an extracellular factor called Pikt, with a molecular weight of 3 kDa. Studies have shown that this potential biopreservative is highly effective against various strains of *D. bruxellensis and S. cerevisiae* DBPVG 6500, a reference strain for killer toxin sensitivity [4].Therefore, in the present study we evaluate the functional properties of a peptide fraction derived from *W. anomalus* metabolism, ranging from 2-10 kDa, namely their antimicrobial activity against foodborne pathogens and their potential as natural antioxidants. By exploring the use of natural preservatives like these bioactive peptides, the food industry can develop healthier and "clean label" products while maintaining safety and extending the shelf life of food items.

METHOD

Production of the 2–10 kDa Fraction:
Wickerhamomyces anomalus DBVPG 3003, from the University of Perugia's yeast collection, was grown in YEPD medium (yeast extract, peptone, glucose) at pH 4.5, 25 °C, and 150 rpm for 4 days. After centrifugation, the supernatant was filtered (0.45 µm, then 0.22 µm), and peptides were concentrated using ultrafiltration with 10 kDa and then 2 kDa membranes, resulting in a 2–10 kDa peptide fraction.

Antimicrobial Assays:
The minimal inhibitory concentration (MIC) was tested using broth microdilution in 96-well plates. The peptide fraction (2 mg/mL in 8% ethanol) was serially diluted and tested against *Candida albicans*, *C. krusei*, *L. monocytogenes*, *Salmonella sp.*, and *E. coli*. Controls included ethanol, chloramphenicol (bacteria), and natamycin (yeasts). Microplates were inoculated with 10^6 cells/mL and incubated (37 °C for bacteria, 30 °C for yeasts). MIC was visually determined as the lowest concentration with no visible growth. Microbial viability was confirmed by plating on appropriate agar media and counting CFUs after 1–2 days.

Antioxidant Activity Assays:
FRAP and DPPH assays were used to evaluate antioxidant activity. In FRAP, the sample's ability to reduce Fe^{3+} to Fe^{2+} was measured at 595 nm. In the DPPH assay, the reduction of DPPH radical absorbance at 515 nm was assessed after 45 minutes at 30 °C. Both used TROLOX standards (0–2000 µM), and results were expressed in µM.

RESULTS & DISCUSSION

Antimicrobial and antioxidant effect exerted by peptides produced by *W. anomalus*

Fungistatic and bacteriostatic effect against foodborne pathogens

The results showed that the tested microorganisms demonstrated normal growth in the absence of the 2-10 kDa peptide fraction and in the presence of ethanol (negative control). The MIC for all bacteria tested was determined to be 0.5 mg/mL (Table 1). However, both *C. albicans* and *C. krusei* displayed resistance to the 2-10 kDa peptide fraction, even at concentrations higher than 1 mg/mL (Table 1). In the case of *E. coli*, the presence of the 2-10 kDa fraction at 1 mg/mL resulted in a significant reduction in the number of initial colony-forming units (CFUs), with an approximate decrease of 5 orders of magnitude comparing to the control (Figure 1). Similarly, *L. monocytogens* and *Salmonella* sp. exhibited reductions of 4 orders of magnitude comparing o the control (Figure 1), indicating that the peptide fraction at 1 mg/mL possessed bactericidal activity against all three bacterial species. At a lower concentration of 0.5 mg/mL, there was a slight reduction in CFUs for *E. coli*, suggesting a bactericidal effect. However, for *Salmonella* sp. and *L. monocytogens*, the peptide fraction exerted only a bacteriostatic effect on these two pathogens (data not shown).

Table 1. Minimum inhibitory concentration (MIC) of 2-10 kDa fraction against the microorganisms tested

Microorganisms	MIC (mg/mL)
Candida albicans	>1
C. krusei	>1
E. coli	0.5
L. monocytogenes	0.5
Salmonella sp.	0.5

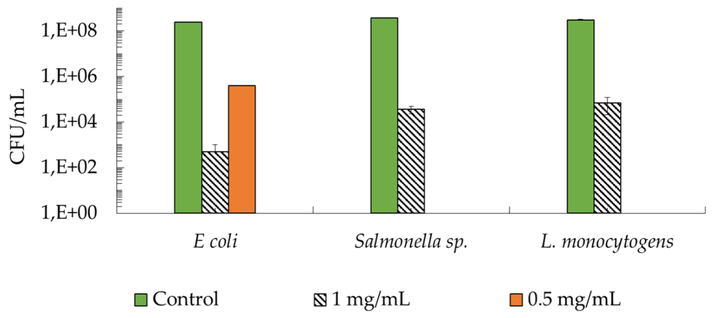
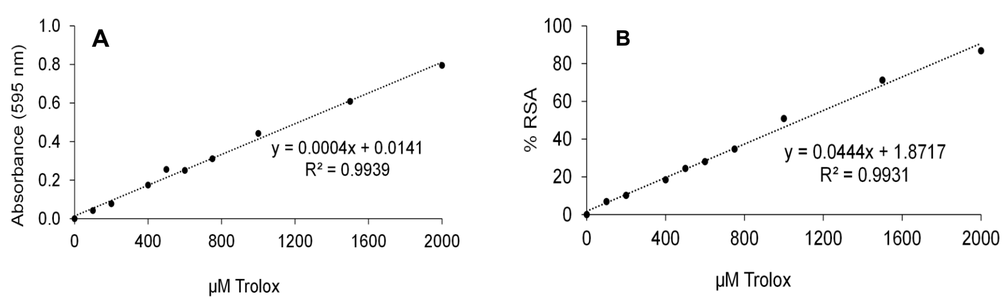


Figure 1. Viability (CFU/mL) of *E. coli* ATCC 25922, *L. monocytogenes* ISA 4008, and *Salmonella* sp. ISA after 24 hours of exposure to the 2–10 kDa peptide fraction at final concentrations of 1 mg/mL and 0.5 mg/mL. A negative control assay (4% v/v ethanol without the peptide fraction) was also included. Data represent the mean ± standard deviation (SD) of two independent biological replicates.

Antioxidant activity

The 2-10 kDa peptide fraction at 1.0 mg/mL exhibited the highest capacity to inhibit the DPPH radical, with a value of 1856 ± 37.5 µM TE/mL, while at 0.5 mg/mL, the inhibition capacity was measured as 1452 ± 400 µM TE/mL (Figure 2C). In the FRAP assay, which measures the reduction of Fe3+ to Fe2+, the 2-10 kDa fraction at 1.0 mg/mL demonstrated a significant capacity, with a value of 5843 ± 220 µM TE/mL (Figure 2C). These results indicate the strong antioxidant potential of the 2-10 kDa fraction.



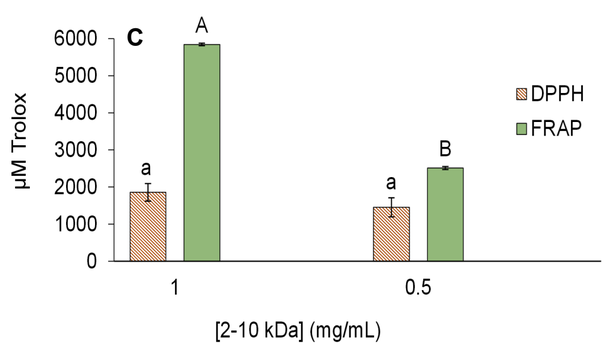


Figure 2 – Calibration curve and equation of the FRAP and DPPH method (A). Values obtained for FRAP and DPPH (B), for the 2-10 kDa fraction at 1 and 0.5 mg/mL (C). Different letters represent significant differences ($p < 0.05$).Antioxidant activity was expressed to equivalent content of µM Trolox.

CONCLUSION

This study demonstrates that the peptides secreted by *W. anomalus* exhibit a clear bactericidal effect against *E. coli*, *L. monocytogenes*, and *Salmonella* sp., significantly reducing their viability after 24 hours of exposure. The antimicrobial activity observed suggests that the 2–10 kDa peptide fraction interferes with the growth and survival of both Gram-negative and Gram-positive bacteria, indicating its broad-spectrum potential. In addition to its antimicrobial properties, the peptide fraction also showed strong antioxidant activity, as confirmed by both FRAP and DPPH assays. These dual bioactivities highlight the potential application of *W. anomalus*-derived peptides as natural preservatives or functional ingredients in food, pharmaceutical, or biomedical industries. Further studies are needed to characterize the specific peptides responsible and to assess their stability and safety in real-world applications.

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