ECA 2025 Conference

The 4th International Electronic Conference on Antibiotics

21–23 May 2025 | Online

MDPI

Evaluation of yeast metabolites as natural preservatives for cosmetic formulations

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INTRODUCTION & AIM

In recent years, consumers have shown a growing preference for cosmetics that are both natural and environmentally sustainable. This shift in demand has driven research into alternatives to synthetic preservatives, which are often linked to negative health outcomes such as allergic reactions and long-term environmental impacts due to their chemical persistence [1]. Although synthetic agents like parabens and formaldehyde releasers remain widely used due to their effectiveness, ongoing safety concerns and tighter regulations have motivated the search for more natural preservation methods [2].

Cosmetic products, by their very nature, are prone to microbial contamination. Their nutrient-rich composition often including water, proteins, and lipids creates an ideal environment for microbial growth. Contamination may arise during production, packaging, or everyday use, increasing the risk of spoilage or even infections. Frequently encountered contaminants such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* are known to compromise both product safety and shelf life [3].

One of the most promising categories of natural antimicrobial agents is antimicrobial peptides (AMPs). These are short proteins naturally produced by a wide variety of organisms, from bacteria to yeasts, and are capable of fighting a broad range of microbes by damaging their membranes or disrupting key cellular functions [4]. Certain yeasts, commonly referred to as "killer yeasts", including *Saccharomyces cerevisiae*, *Wickerhamomyces anomalus,* (formerly known as *Pichia anomala)* and *Tetrapisispora phaffii*, are known for producing AMPs that inhibit competing microorganisms, making them attractive candidates for natural preservation strategies [5-7] Integrating those yeast peptides into cosmetic products could provide a safer, more eco-friendly alternative to conventional preservatives, while also meeting rising consumer expectations and regulatory standards.

Therefore, this project aims to evaluate the antimicrobial potential of peptide fractions produced by *S. cerevisiae*, *W. anomalus*, and *T. phaffii* as natural preservatives in a cosmetic cream formulation, targeting the inhibition of common pathogenic microorganisms.

METHOD

W. anomalus NCYC 434 and *T. phaffii DBVPG 6076* were grown in YEPD medium supplemented with 50 g/L glucose (pH 4.5). *S. cerevisiae* ISA 1028 was cultured in synthetic must medium, containing glucose and fructose (110 g/L each), and adjusted to pH 4.5. All strains were incubated at 28 °C, 150 rpm for 4 days.

After 4 days, cultures were centrifuged and filtered to obtain cell-free supernatants. Peptides were then fractionated and concentrated using Amicon® ultrafiltration units into three molecular weight ranges: 2–10 kDa for *W. anomalus and S. cerevisiae* and >10 kDa for *T. phaffii*. The antimicrobial activity of peptide fractions against common cosmetic contaminants was evaluated using the broth microdilution method in 96-well plates, following the protocol described by Pereira et al. (2022) [8].

Minimum bactericidal concentration (MBC) assays were then performed to determine the bactericidal potential of the active fractions.

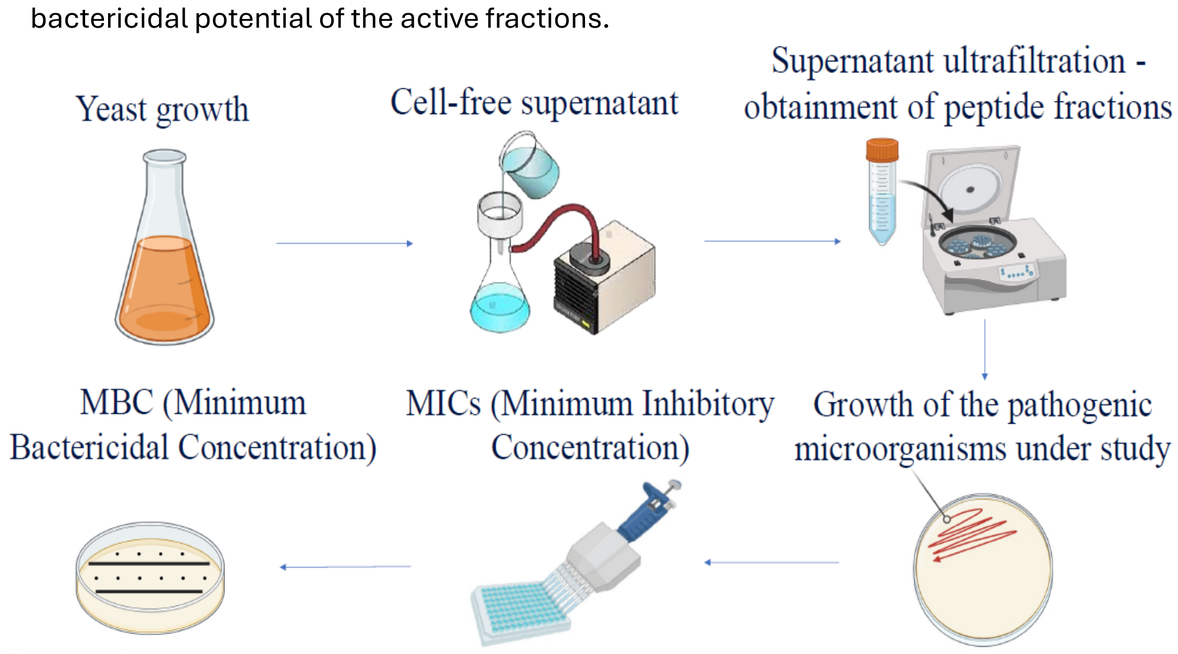

Figure 1: Schematic methodology

RESULTS & DISCUSSION

The quality control of the cosmetic cream formulation showed satisfactory physicochemical properties, with stable pH (5.27), no phase separation, and appropriate viscosity (18,000 sp5/20 rpm). Microbiological tests confirmed the absence of contamination (data not shown).

Minimum Inhibitory Concentration (MIC) results (Table 1) revealed promising antimicrobial activity for several peptide fractions. Particularly, the 2–10 kDa fraction from *S. cerevisiae* was the most effective, with low MIC values against *Staphylococcus aureus* (56.25 µg/mL), *Escherichia coli* (225 µg/mL), and *Bacillus cereus* (225 µg/mL). The >10 kDa fraction from *W. anomalus* also showed significant activity, especially against *Methicillin-resistant Staphylococcus aureus (MRSA)* and *Streptococcus epidermidis* (450–575 µg/mL). However, fractions from *T. phaffii* showed generally higher MICs, indicating lower efficacy.

Table 1: Minimum Inhibitory Concentration (MIC) of peptide fractions derived from *S. cerevisiae, W. anomalus* and *T. phaffii metabolism* against common microbial contaminants found in cosmetic products

	(Minimum Inhibitory Concentration) (µg/ mL)			
Microorganism	2-10 kDa fraction *S. cerevisiae*	2-10 kDa fraction *W. anomalus*	*T. phaffii* >10 kDa fraction	Positive Control[a]
B. cereus	225	575	1250	1.563
S. epidermidis	900	575	2500	0.781
S. aureus	56.25	143.75	156.25	0.781
MRSA	900	575	1250	0.781
P. aeruginosa	900	2300	2500	50
E. coli	225	1150	1250	6.250
S. mutans	900	1150	1250	0.781
C. albicans	1800	287.5	1250	0.039

[a] ketoconazole for *C.albicans*; vancomycin for *B.cereus*, *S.epidermidis, S.mutans, S.aureus and* MRSA ; chlorophenicol for and *E.coli; P.aeruginosa.*

Minimum Bactericidal Concentration (MBC) results (Figure 2) confirmed that several peptide fractions had bactericidal effects, particularly the 2–10 kDa fraction from *S. cerevisiae*, which was active against *B. cereus and S.epidermidis*.

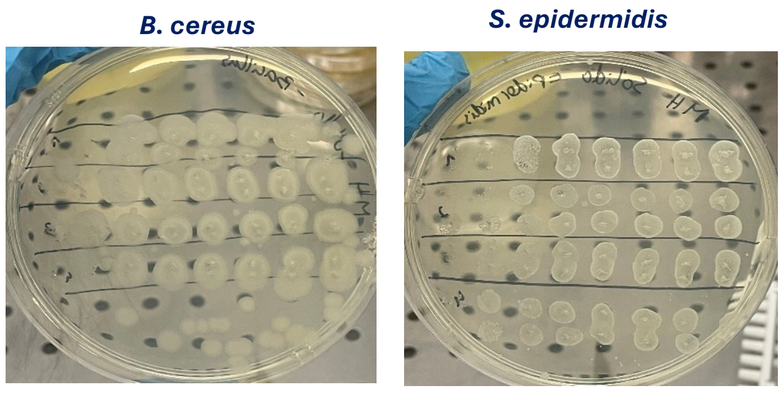

Figure 2: Minimum Bactericidal Concentration (MBC) assays upon addition of the 2–10 kDa fraction from *S. cerevisiae* against *B. cereus and S. epidermidis*.

CONCLUSION

The peptide fractions, particularly the 2–10 kDa from *S. cerevisiae* and the >10 kDa from *W. anomalus,* show promising antimicrobial and bactericidal activity against several common cosmetic contaminants, especially Gram-positive bacteria.

These results support the potential use of yeast-derived peptides as natural preservatives in cosmetic formulations.

FUTURE WORK

To validate these findings and move toward practical application, future studies should:

- Perform the challenge test to the full 28-day period as defined by ISO 11930.
- Test additional cosmetic formulations to evaluate performance across product types.
- Investigate the stability of peptides in cosmetic matrices over time.
- Explore synergistic effects between peptide fractions and conventional preservatives

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