Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente, Ciclo XXXIX

Identification and characterization of new antimicrobial compounds in yeasts

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Background and projectual idea

Food-borne illness (FBI) is caused by the ingestion of foods and/or beverages that have contaminants capable of compromising quality. FBI may also compromise the health of the consumer when is present in sufficient quantities. Close to 250 agents that cause FBI and present different symptomologies have been described. These agents include bacteria, viruses, fungi, and metals, among others. Over the past decade, natural products have been heavily relied upon as sources of therapeutic agents, with antimicrobials being one of the most compelling biomolecules. In particular, they constitute more than two-thirds of newly approved medicinal products used for pharmaceutical applications.

Abstract

This study aims to characterize yeasts isolated from various sources such as fruits, cheese, malt, and other anthropized environmental for their antimicrobial activity against pathogens (such as bacteria, molds and/or other yeasts). A general screening test for the expression of antimicrobial activity was performed on over 104 cultures belonging to 12 yeast genera. Of these cultures, 4 *Brettanomyces*, 1 *Debaryomyces*, 3 *Hanseniaspora*, 3 *Metschnikowia*, 4 *Pichia*, 7 *Saccharomyces*, 21 *Torulaspora* e 5 *Meyerozima* were found to produce zones of inhibition of bacterial or yeast growth on well test Agar medium supplemented with 0.002% methylene blue. Of 4 bacteria and 3 yeasts used as test organisms, only 3 bacteria and all 3 yeasts were inhibited.
A second step involves studying the molecules responsible for the inhibition process. Inhibition can be caused by the production of peptides, glycoproteins, or metabolites, or through cell-cell contact and quorum sensing interactions.

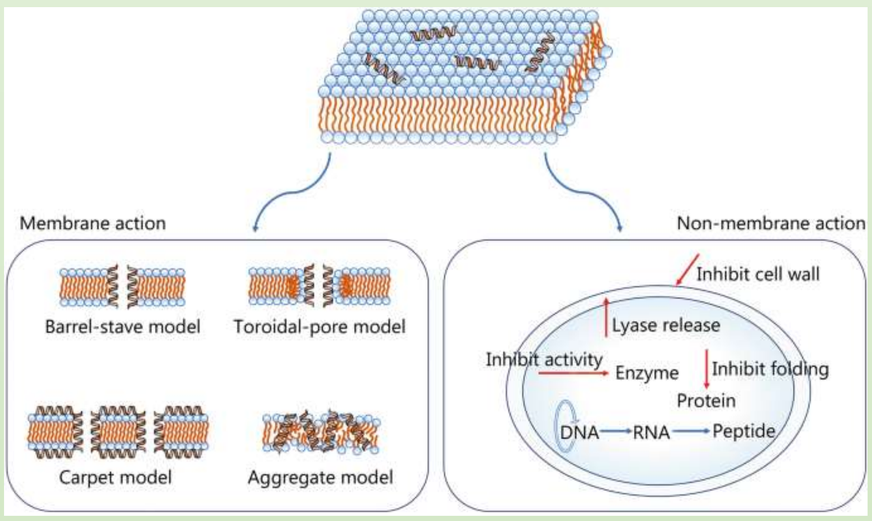
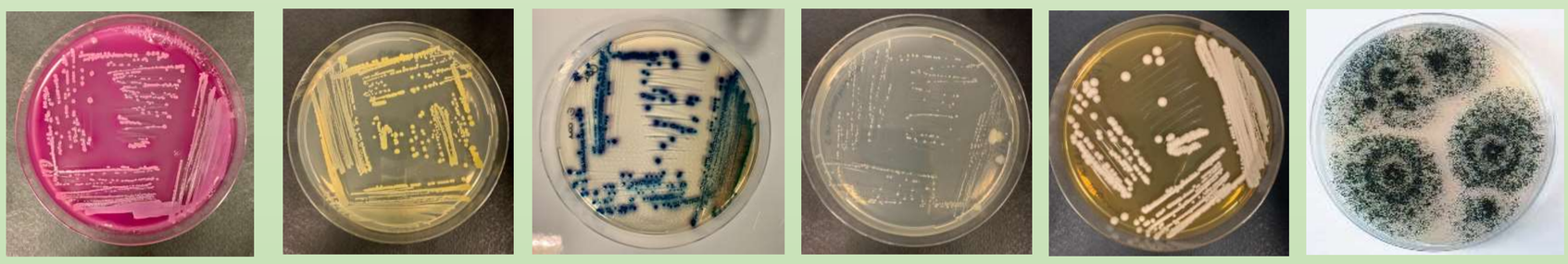


Fig. 1 Models of antibacterial mechanisms of AMPs. The direct bactericidal mechanism of AMPs is performed through interacting with negatively charged membranes, resulting in increased membrane permeability, cell membrane lysis, or release of intracellular contents, which ultimately leads to cell death. Another bactericidal mechanism is that AMPs penetrate into the cytoplasm and interact with intracellular substances, such as inhibiting DNA, RNA and protein synthesis, inhibiting protein folding, inhibiting enzyme activity and cell wall synthesis, and promoting the release of lyases to destroy cell structures. AMPs antimicrobial peptides

Methods

Agar well diffusion against emergent pathogens in sanitary and food industry environment.
Yeasts with 48h of advantage, were brought in contant with bacteria, forming a bilayer, for 18h at 37°C. Results were evaluated by measuring the diameter of the inhibition halo.



Salmonella enteritidis | *Staphylococcus aureus* | *Escherichia Coli* | *Listeria monocytogenes* | *Candida albicans* | *Aspergillus niger*

Figure 2. Pathogens on plate: from right *S. enteridis, S. aureus, E. Coli, L. monocytogenes, C. albicans, A. niger.*

Preliminary results

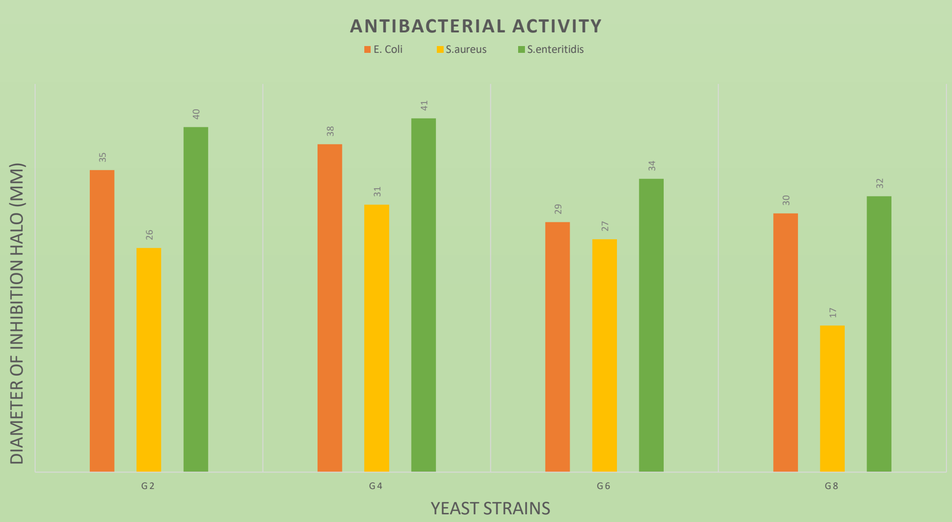
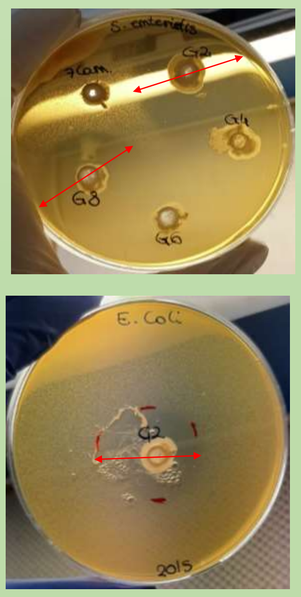


Figure 3. This graphic shows four strains of the Brettanomyces genus. For each strain, the diameter of the inhibition halo against E. coli (orange), S. aureus (yellow), and S. enteritidis (green) is represented. On the right, the plates with the tested strains are shown.

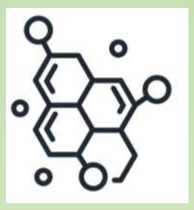


The results obtained from the initial screening against some bacteria are encouraging. Some yeast strains, particularly those isolated from fruits, show a high capacity to inhibit emerging pathogenic bacteria such as *E. coli*, *S. aureus*, and *S. enteritidis*.
E. coli and *S. enteritidis*, as reported in various studies, are two of the principal agents responsible for a high number of reported cases of foodborne infection.
Particularly interesting results are shown by the strain "2A" of *Metschnikowia pulcherrima,* this strain has inhibitory activity against 3 bacteria and 2 yeasts . As reported by L. Oro et al. the antimicrobial activity of *M. pulcherrima* does not seem due to proteinaceous compounds such as killer phenomenon, but to the pulcherriminic acid (the precursor of pulcherrimin pigment) the depletes iron present in the medium, making it not available to the other yeast.

Future prospects after antimicrobial characterization

Active biopackaging

Sanitary application



Probiotic

Food industry

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Acuña-Fontecilla, A., et al., Evaluation of antimicrobial activity from native wine yeast against food industry pathogenic microorganisms. CyTA - Journal of Food,2017.
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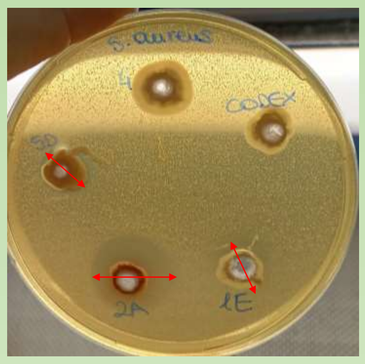

Figure 4. The strains 2A *M. pulcherrima* tested against *S. aureus.* The diameter of inhibition halo is highlighted by an arrow