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

Identification and characterization of new antimicrobial compounds in bioactive yeasts

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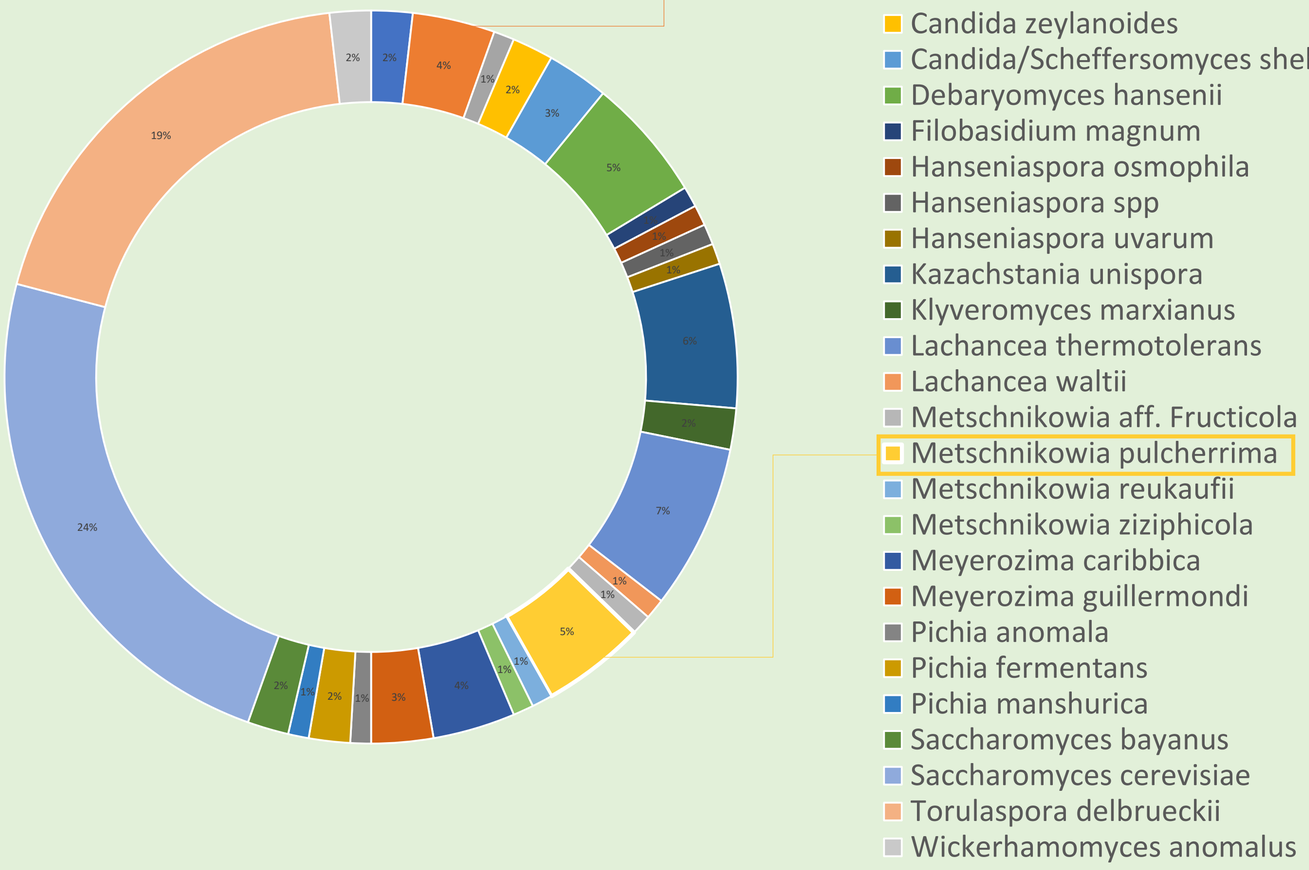
Background and objective

In recent decades, increasing concerns about food safety and the need to reduce the use of chemical preservatives have driven interest toward natural biocontrol strategies. Among these, the use of antagonistic microorganisms—particularly yeasts—has shown promise in counteracting the growth of spoilage or pathogenic microorganisms, including antibiotic-resistant bacteria and toxigenic molds. Many yeasts are known to produce secondary metabolites (such as killer toxins, organic acids, and volatile compounds) with antimicrobial activity. Some species belonging to the genera *Saccharomyces*, *Metschnikowia*, *Pichia*, and *Candida* have been studied for their ability to inhibit the growth of phytopathogenic molds or human pathogens, offering a valid alternative or complement to conventional antimicrobial agents. However, the specific mechanisms of action, the spectrum of microbial targets, and the optimal conditions to maximize the antimicrobial effect of yeasts remain insufficiently explored—especially in complex environments such as food systems. This has led to growing interest in systematically evaluating the antimicrobial activity of various yeast strains against molds and pathogenic bacteria, with the aim of selecting promising strains for use in biocontrol or as biopreservatives.

Materials



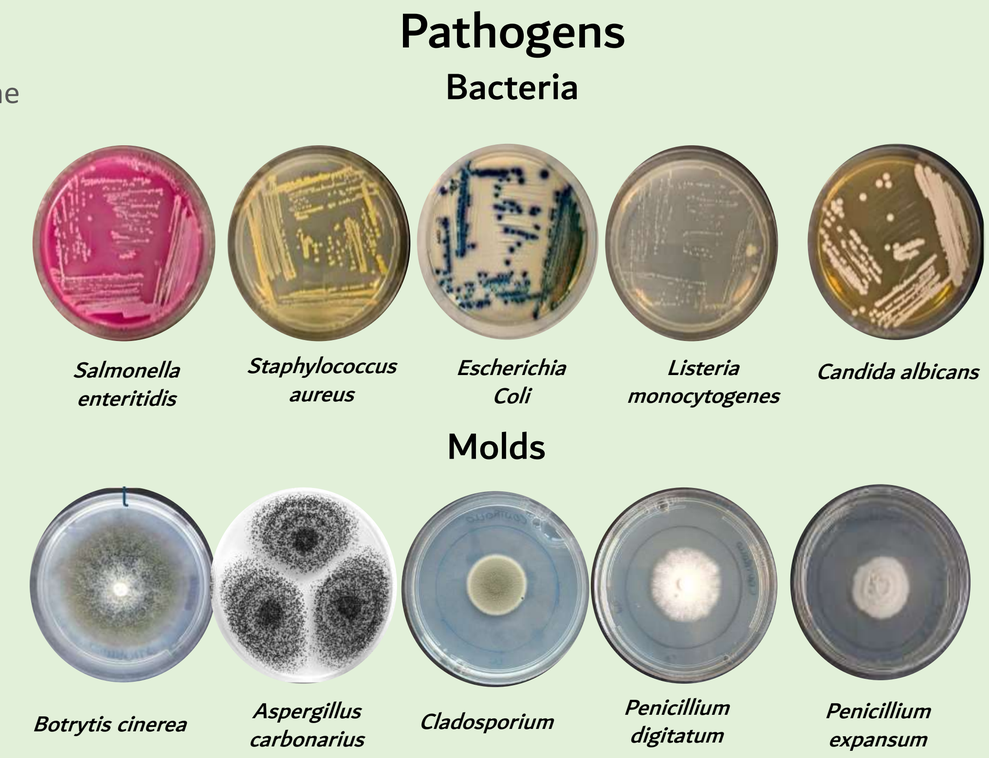


Fig. 1 On the left yeast species selected for in vitro screening for inhibitory activity against pathogens (on the right)

Methods

Evaluation of inhibitory activity against pathogen molds and bacteria. In vitro screening of a wide range of yeast strains belonging to 21 different species.

Metabolites assay Pathogen and yeast put in touch on the same plate

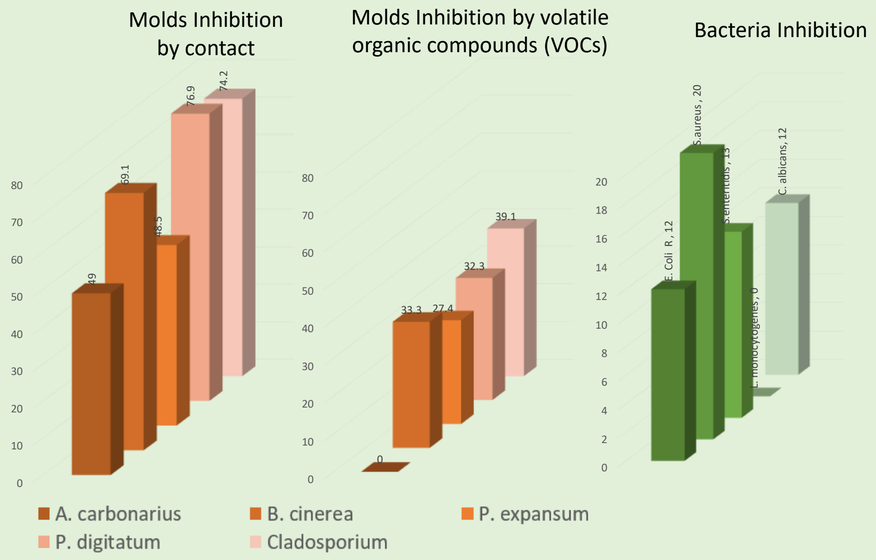
VOCs assay Pathogen and yeast in dual plate assay for VOC inhibitory activity evaluation

Results

Fig. 2 Screening – Best 20 inhibition activities against molds

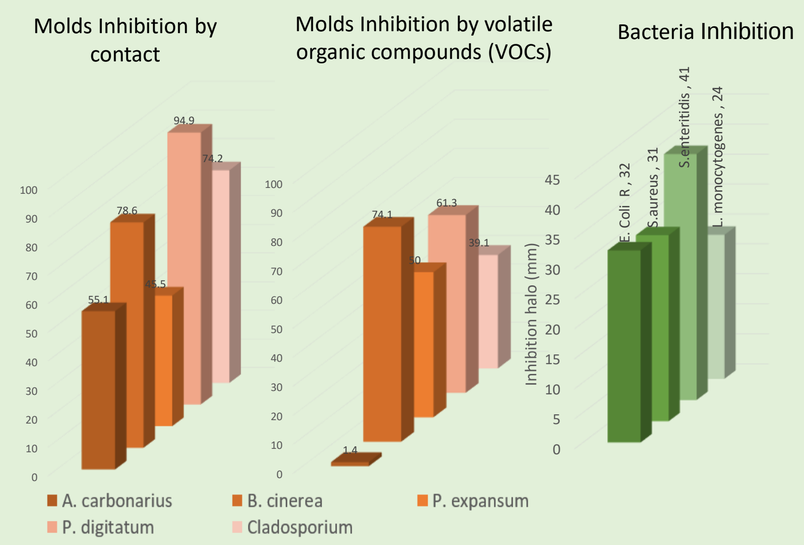
A. carbonarius		*B. cinerea*		*Cladosporium*		*P. Expansum*		*P. Digitatum*	
Na32	69.4%	G2	100%	1E	80.7%	G2	48.5%	G4	94.9%
G4	55.1%	G6	82.1%	G8	77.4%	2A	48.5%	G8	92.3%
2A	49%	40	82.1%	G4	74.2%	G4	45.5%	G2	84.6%
G8	44.9%	m1-2	81%	2A	74.2%	G6	39.4%	40	82%
G6	42.9%	m1-6	79.8%	103	74.2%	46 Mp	39.4%	2A	76.9%
G2	42.9%	LV8	79.8%	m3-7	71%	B42	36.4%	PC5_1165	74.4%
103	40.8%	G4	78.6%	46 Mp	71%	Na32	33.3%	B42	71.8%
m3-B3	34.7%	28	77.4%	102	71%	m3-5	33.3%	G6	66.7%
28	34.7%	1E	77.4%	40	64.5%	B27	33.3%	46 Mp	66.7%
1E	34.7%	m1-8	76.2%	m3-4	61.3%	1E	33.3%	m3-7	64.1%
m3-7	32.6%	M1_1175	76.2%	m1-9	61.3%	103	33.3%	10C	61.5%
m3-5	32.6%	Mc95	75%	m1-7	61.3%	Ma	30.3%	m3-C3	59%
m3-4	32.6%	B8	75%	m1-6	61.3%	m3-B3	30.3%	BEM1_1175	59%
m1-8	32.6%	19.3t2	73.8%	m1-3	61.3%	m3-A3	30.3%	m3-6	56.4%
B42	32.6%	Na32	72.6%	m1-2	61.3%	m3-6	30.3%	B6	56.4%
92	32.6%	LV12	71.4%	G6	61.3%	LV12	30.3%	m3-4	53.9%
5	32.6%	B42	69%	15.2t2	61.3%	G8	30.3%	92	53.9%
46 Mp	32.6%	B27	69%	m3-5	58.1%	B49	30.3%	8	53.9%
40	32.6%	2A	69%	m1-1	58.1%	B28	30.3%	3003	53.9%
m1-9	30.6%	37	67.9%	6809	58.1%	18 Pi.	30.3%	m3-5	51.3%
Botector	10.2%	Botector	56%	Botector	9.7%	Botector	24.2%	Botector	35.9%

Fig. 3 Metschnikowia pulcherrima stranis 2 A - Inhibitory activity



Action mediated probably by metabolites

Fig. 4 Brettonomyces bruxellensis stranis G4 - Inhibitory activity



Action mediated also by Volatile organic compounds

Initial *in vitro* screening yielded promising results, particularly for four strains of *Brettanomyces bruxellensis*—strains G2, G4 (Fig. 4), G6, and G8—which showed a broad spectrum of inhibition against molds as well as pathogenic bacteria and yeasts. In particular, their activity mediated by volatile compounds warrants further investigation.

On the other hand, other strains also stood out for their broad-spectrum antimicrobial effects, such as three strains of *Metschnikowia pulcherrima* (strain "2A" in Fig. 3), a species already well-documented in the literature for its antimicrobial activity mediated by pulcherrimin. Preliminary tests suggest that its action may be more related to compounds produced and released into the medium rather than volatile compounds.

The *in vitro* results were confirmed by preliminary *in vivo* trials conducted on grapes, apples, and strawberries. Further studies are needed to better understand their mechanisms of action and to explore potential future applications.

Bibliography

Bilinski CA, Innamorato G, Stewart GG. 1985. Identification and characterization of antimicrobial activity in two yeast genera. Appl Environ Microbiol 50:.

Nayyereh Alimadadi et al., Screening of antagonistic yeast strains for postharvest control of Penicillium expansum causing blue mold decay in table grape, Fungal Biology,2023