



Grapevine fungal pathogens in post-harvest: new BCAs selected by CREA – Viticulture and Enology (South Italy)

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Co-tutors:

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Dott. Antonio Domenico Marsico

PhD student:

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Annual Grape Losses



Caused →

Fungal pathogens

The main:
B. cinerea



Control →

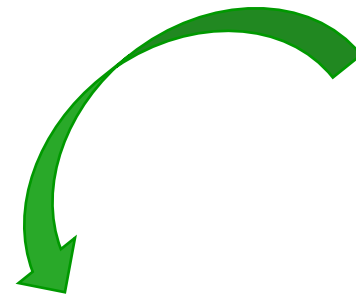
Fungicides
SO₂ generator pads

→

**ANTIMICROBIAL
RESISTANCE**



Secondary fungal
pathogens:
Cladosporium spp,
Penicillium spp,
Alternaria spp,
Aspergillus spp.



NEW ALTERNATIVE STRATEGIES:

**Biological Control Agents
(BCAs)**



Research of *B. cinerea* antagonist microorganisms for table grapes (to replace/supplement existing agrochemicals)

AIM



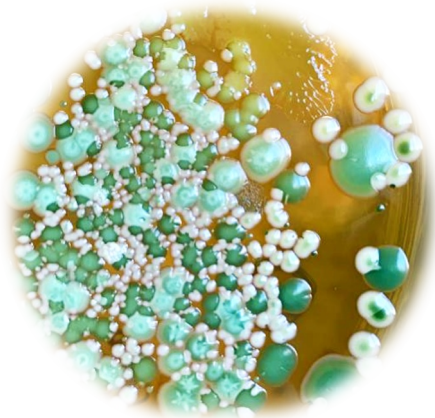
Semi-selective isolation and characterization of non-*Saccharomyces* yeasts as BCAs



Evaluation of antagonistic activity



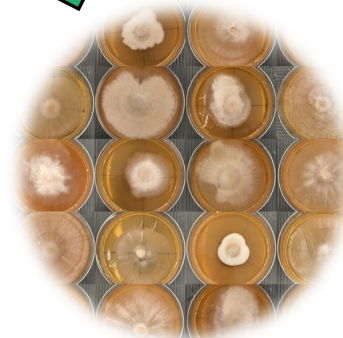
Technological and sustainable development of yeasts



In vivo



In vitro





Research of *B. cinerea* antagonist microorganisms for table grapes (to replace/supplement existing agrochemicals)

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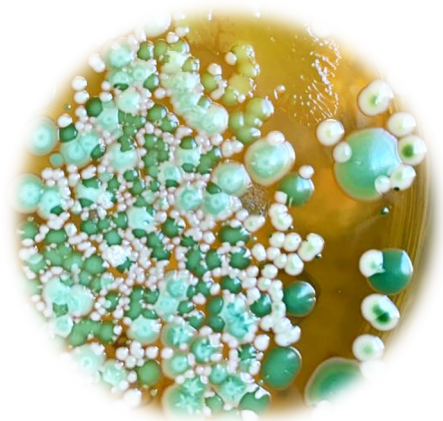
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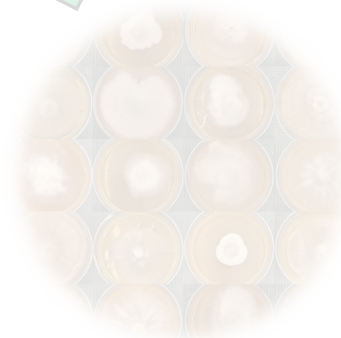
Technological and sustainable development of yeasts



In vivo



In vitro



NEW ISOLATIONS OF NON-SACCHAROMYCES YEASTS

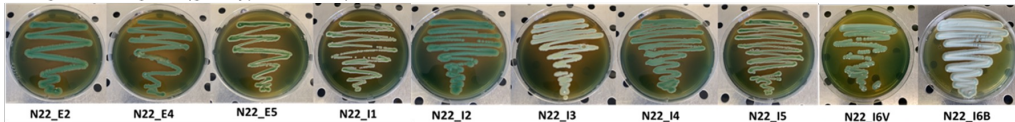
Semi-selective isolation performed on a selection of new *V. vinifera* genotypes, deriving from the CREA-VE Genetic Improvement Program



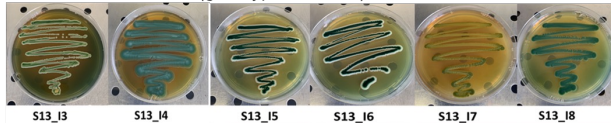
- 7 genotypes with:
- Different degree of tolerance to *B. cinerea*
 - Compact bunch

31 NON-SACCHAROMYCES YEAST ISOLATES

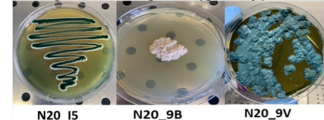
Red globe x Regal B (genotype N22/132)



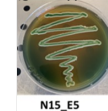
Ceresa Rs x Carati B (genotype S13/158)



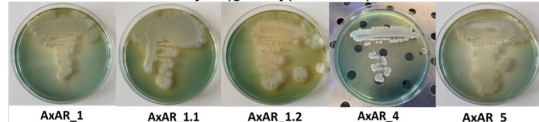
Red globe x Regal B (genotype N20/057)



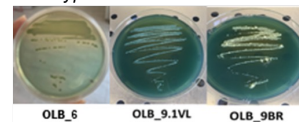
Cimminita B x Melissa B (genotype N15/048)



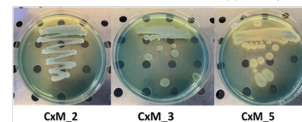
Almeria x Autumn Royal (genotype AxAR)



Genotype Olivetta B



Cimminita B x Melissa B (genotype C x M)



- 1) Morpho-colorimetric on WL and microscopic characterization
- 2) *In vivo* and *in vitro* antagonism test



Research of *B. cinerea* antagonist microorganisms for table grapes (to replace/supplement existing agrochemicals)

AIM



Semi-selective isolation and characterization of non-*Saccharomyces* yeasts as BCAs



Evaluation of antagonistic activity

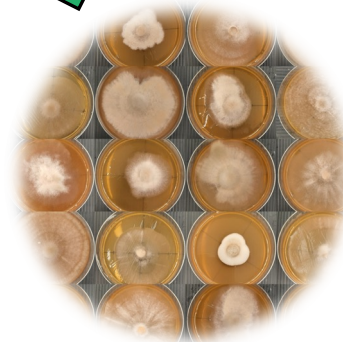
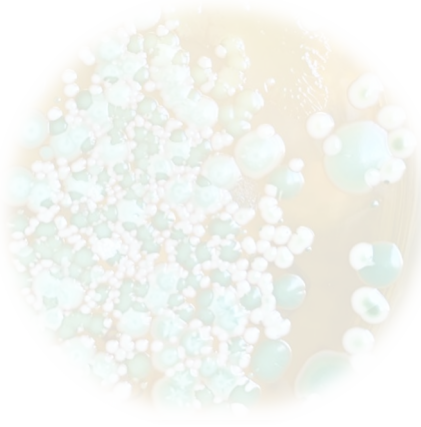


Technological and sustainable development of yeasts

In vivo



In vitro



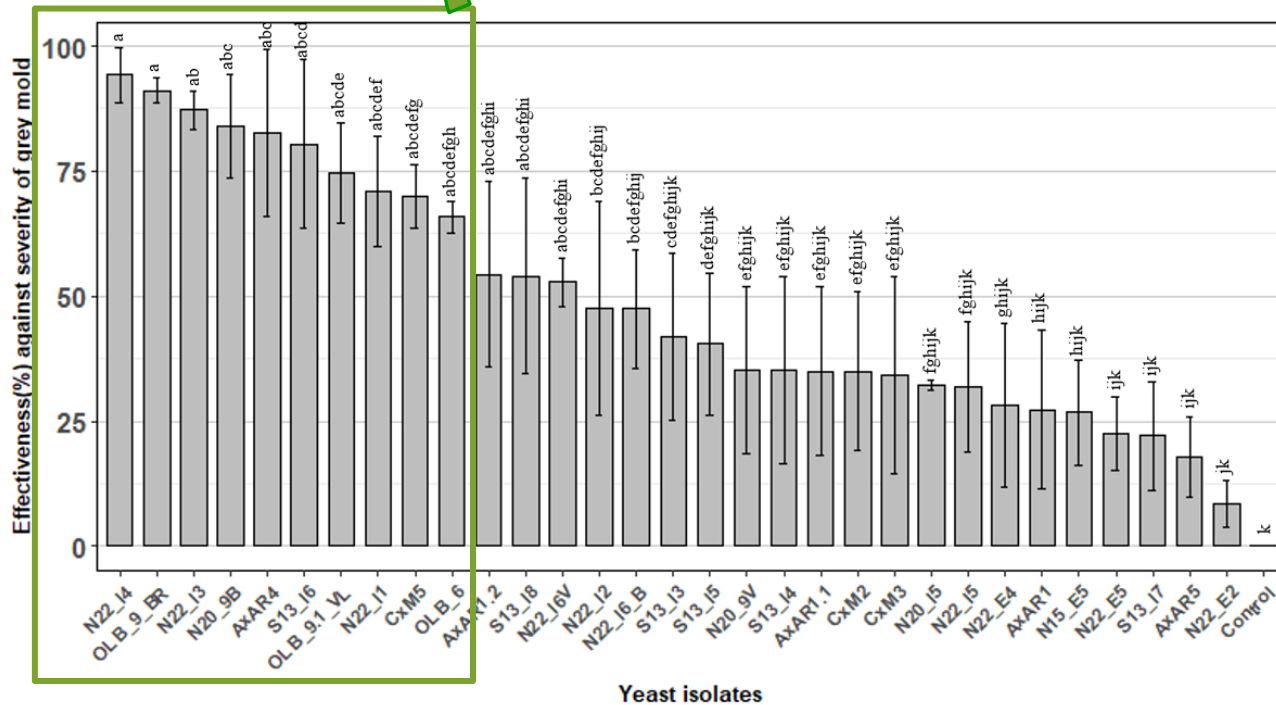
ANTAGONISTIC ACTIVITY OF THE NEW YEASTS

In vivo tests



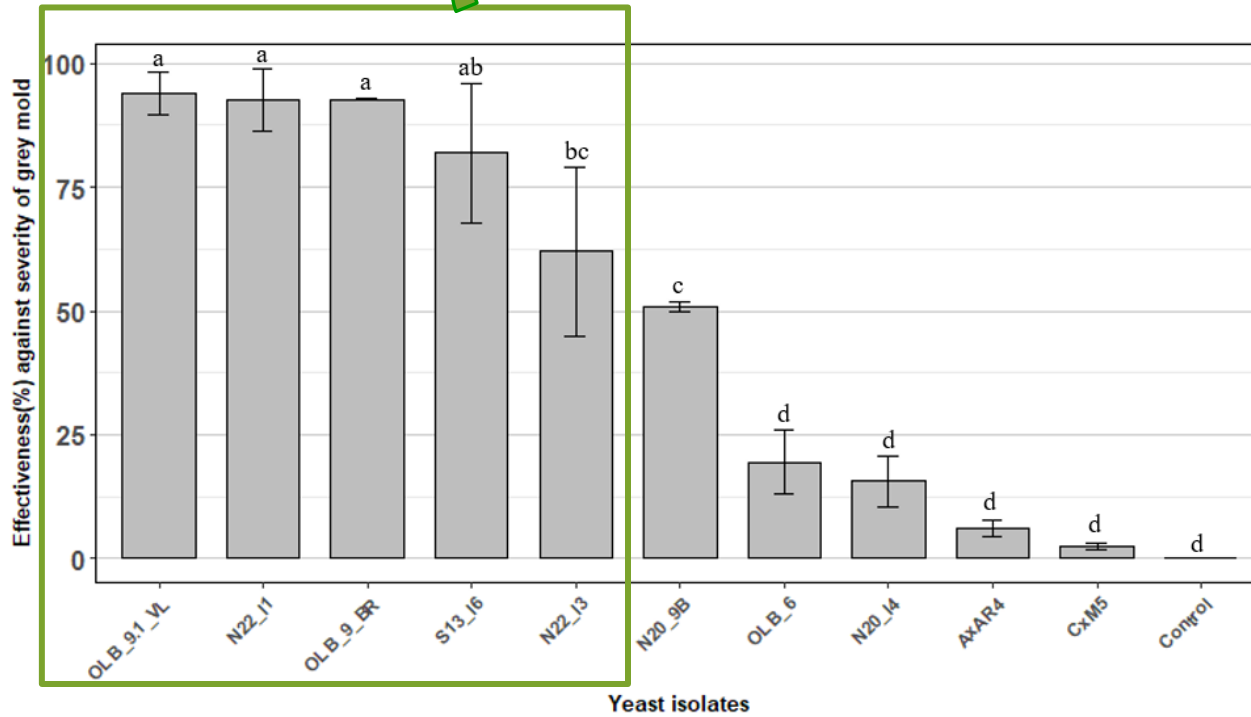
Artificial wounds on sterile grapes, inoculated with each yeast isolate (1.5×10^7 CFU mL⁻¹) and, after 48 h of incubation at 25°C, with a suspension of *B. cinerea* (1×10^5 conidia mL⁻¹)

Effectiveness $\geq 60\%$

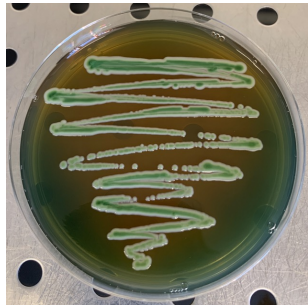


In vivo tests

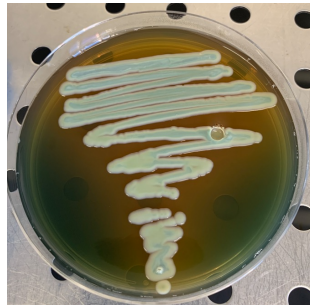
Effectiveness $\geq 60\%$



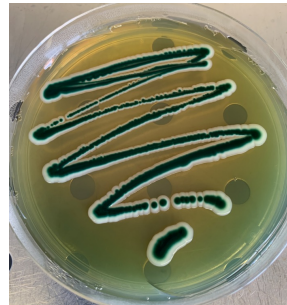
MOLECULAR IDENTIFICATION



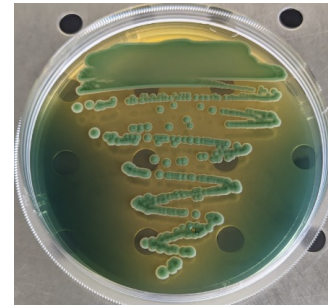
Starmarella bacillaris



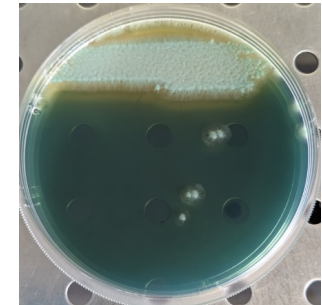
Saturnispora diversa



Starmarella bacillaris



Hanseniaspora uvarum



Aureobasidium pullulans

BIOCONTROL MECHANISMS

Competition for Nutrients and Space



Mycoparasitism



Siderophore



Secreted enzymes



Toxins



Induction of Host Resistance



Production of Volatile Organic Compounds (VOCs)

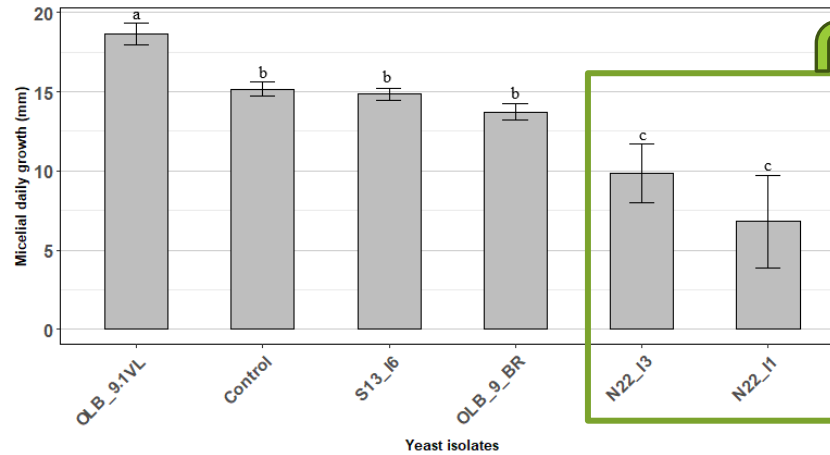
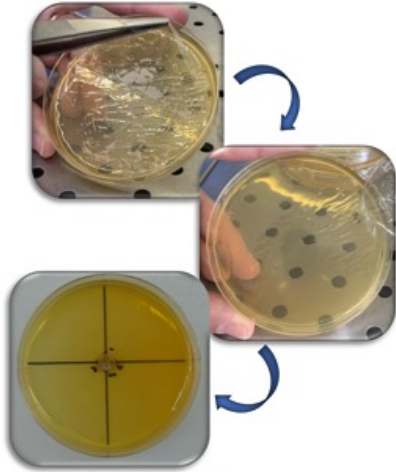


ANTAGONISTIC ACTIVITY OF THE NEW YEASTS

In vitro tests:

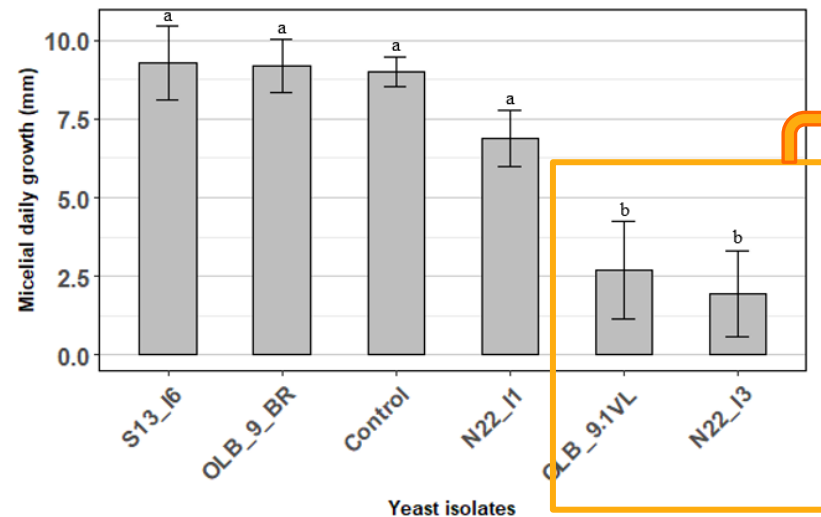
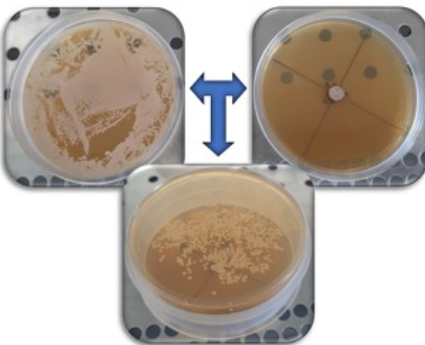
- 1) Cellophane Agar Layer technique (CALt)
- 2) Volatile Organic Compounds (VOCs)
- 3) Extracellular Lytic Enzymatic activities of yeasts

1)



S. Bacillaris N22_I1 and *S. diversa* N22_I3: Production of **diffusible substances** in the medium with fungistatic action

2)

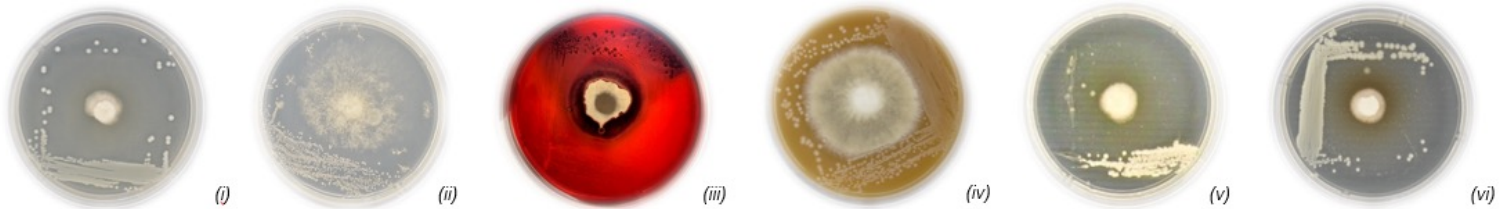


S. diversa N22_I3 and *A. pullulans* OLB_9.1_VL: Production of **volatile substances** with fungistatic action

ANTAGONISTIC ACTIVITY OF THE NEW YEASTS

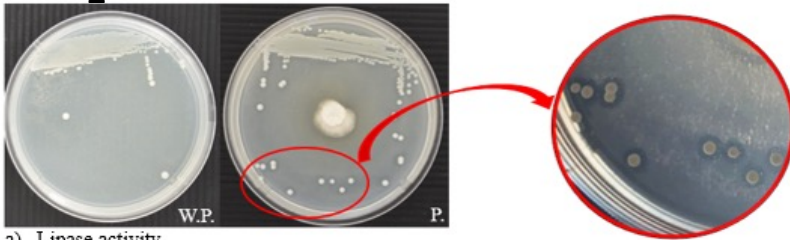
In vitro tests:

3) Extracellular Lytic Enzymatic activities of yeasts



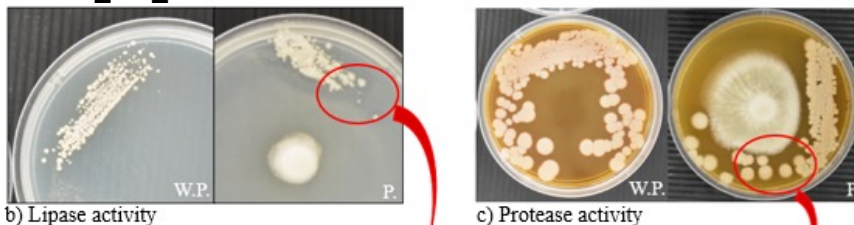
Different media to test the ability to produce enzyme compounds involved in defense against *B. cinerea* by yeasts: lipase (i), chitinase (ii), β -1,3-glucanase (iii), protease (iv), pectinase (v) and esterase (vi) activity.

N22_I3

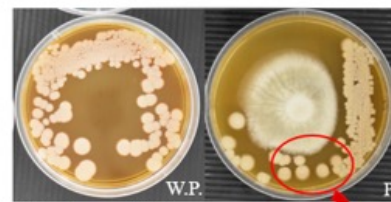


a) Lipase activity

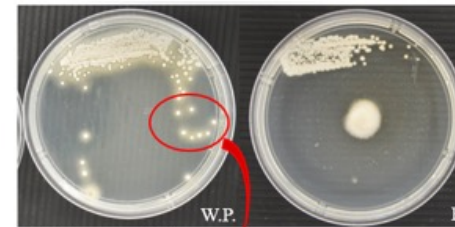
OLB_9.1_VL



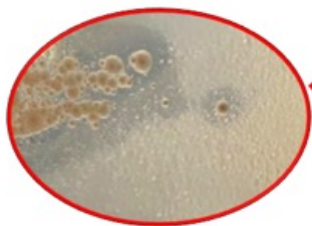
b) Lipase activity



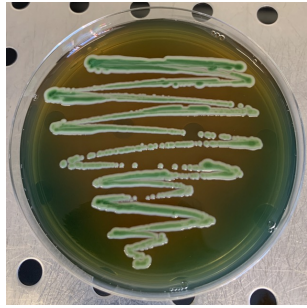
c) Protease activity



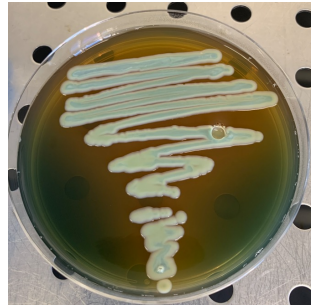
d) Esterase activity



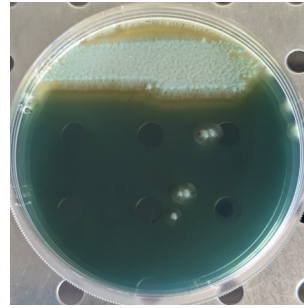
5 new antagonistic yeasts against *B. cinerea*:



S. bacillaris N22_I1



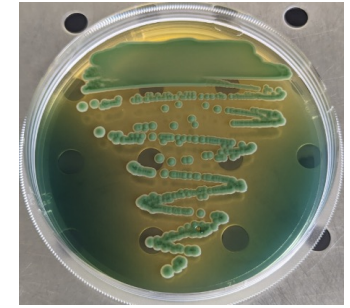
S. diversa N22_I3



A. pullulans OLB_9.1_VL



S. bacillaris S13_I6



H. uvarum OLB_9_BR

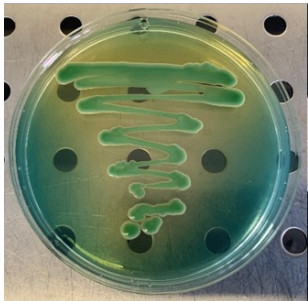
Diffusible substances

Volatile compounds

Competition for Nutrients and Space



4 yeasts (BCAs) already characterized for their antagonistic activity against *B. cinerea* and present in the CREA-VE collection:



L. thermotolerans N10



M. pulcherrima Pr7



M. pulcherrima N20/006



M. pulcherrima Ale4

Diffusible substances

Volatile compounds



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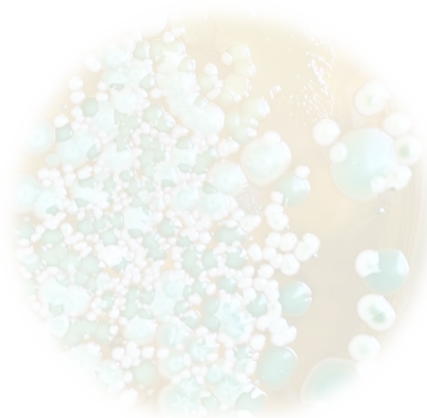
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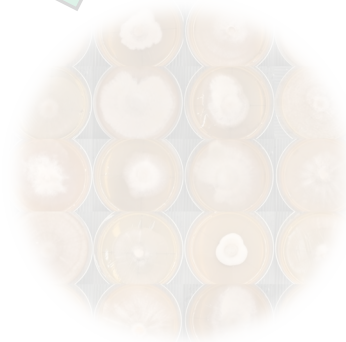
Technological and sustainable development of yeasts



In vivo



In vitro

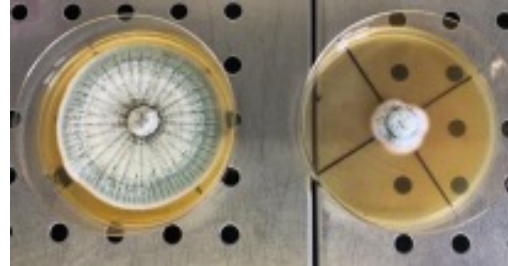


POTENTIAL TECHNOLOGICAL DEVELOPMENT

Characteristics of an ideal biological control agent for commercial development:

- Genetically stable
- Effective at low concentrations
- Non-demanding in terms of required nutrients
- Survival capability under adverse environmental conditions
- Capability of adhering to and colonizing the host surface
- Effective against a range of phytopathogens
- Production in a low-cost growth medium
- Not detrimental to human health
- Environmentally friendly
- Compatible with commercial processing procedures

- Effective at **low concentrations** against a **wide range of pathogens** of grapes



- **Not harmful to human health**: inability to destroy erythrocytes present in blood agar.

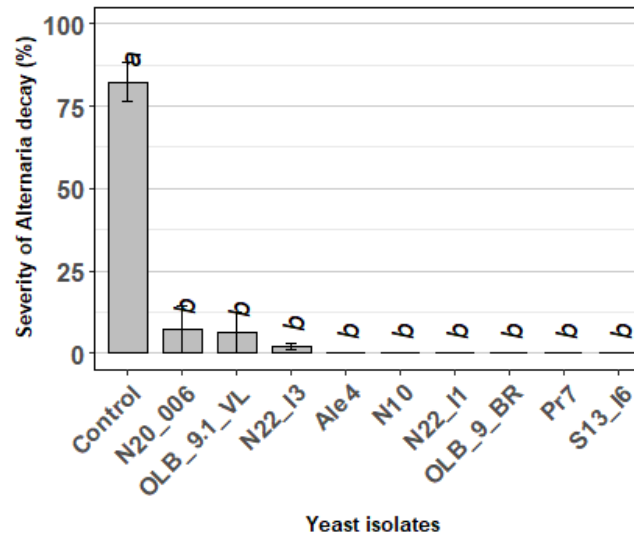
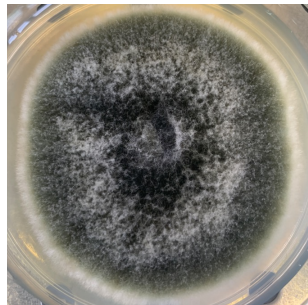
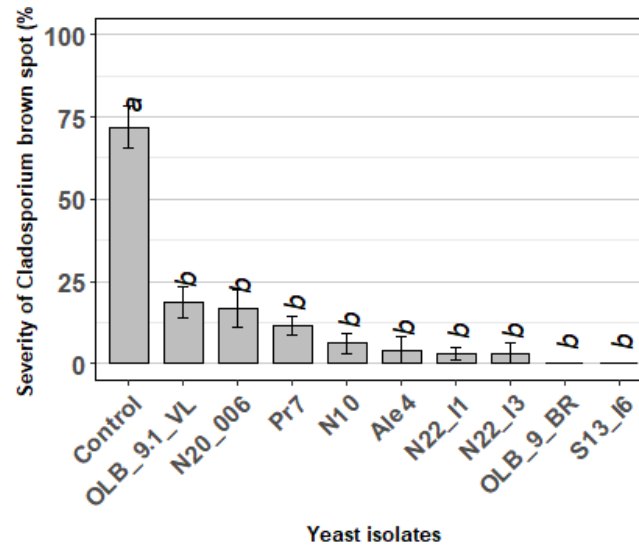


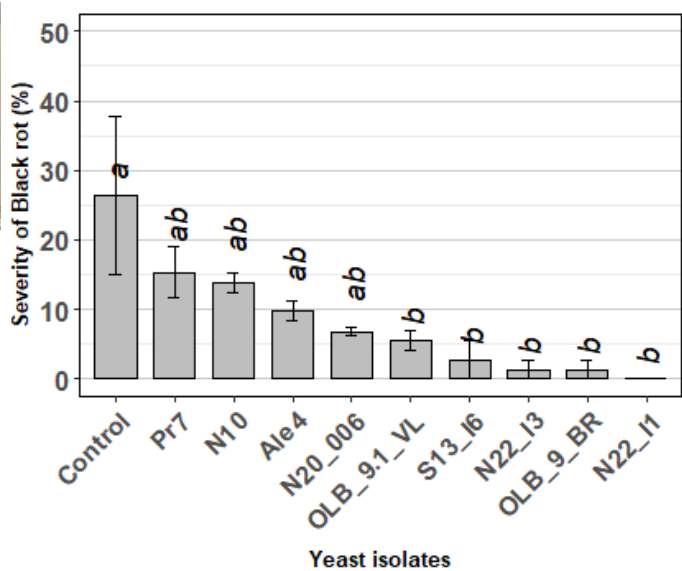
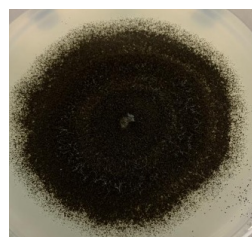
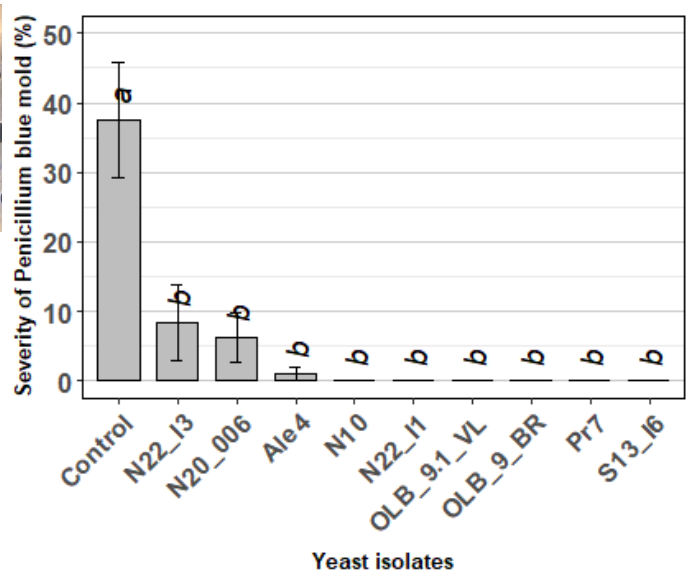
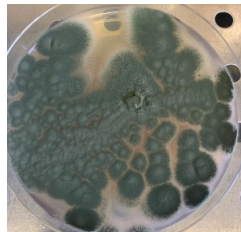
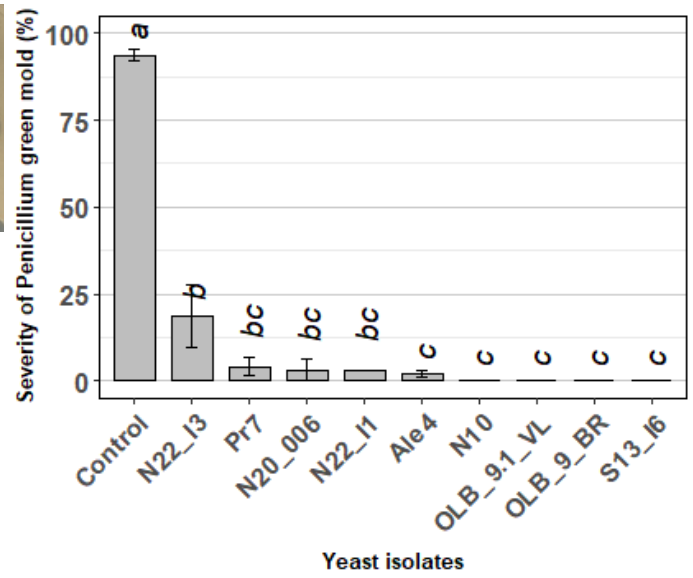
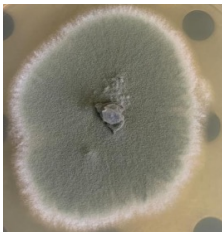
- Ability to survive the **freeze-drying process**, for future commercial use.



➤ Evaluation of antagonistic activity against fungi causative agents of **secondary grape rot** (CALt) at **low concentration** (1.5×10^5 CFU mL⁻¹).

- To verify the efficacy of the yeasts against different targets: *Cladosporium sp*, *Alternaria alternata*, *Penicillium digitatum*, *Penicillium glabrum*, *Aspergillus niger*





➤ Survival to **freeze-drying process**.

- The resistance of yeast strains to the lyophilization and their shelf-life at different storage temperatures (+25°C and +4°C) were evaluated.
- Their viability was evaluated by measuring a survival factor to the lyophilization process (SF_L) and a survival factor to storage after 30 days (SF_S).

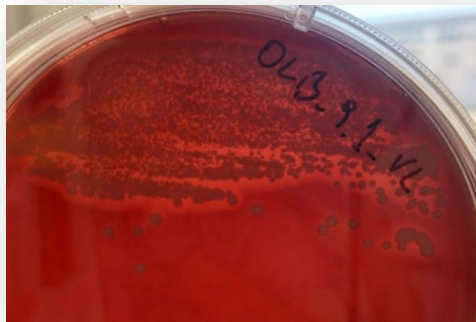
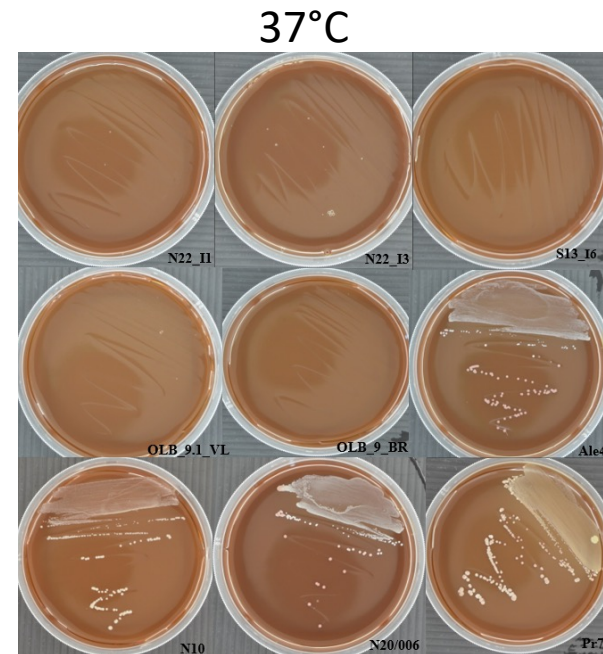
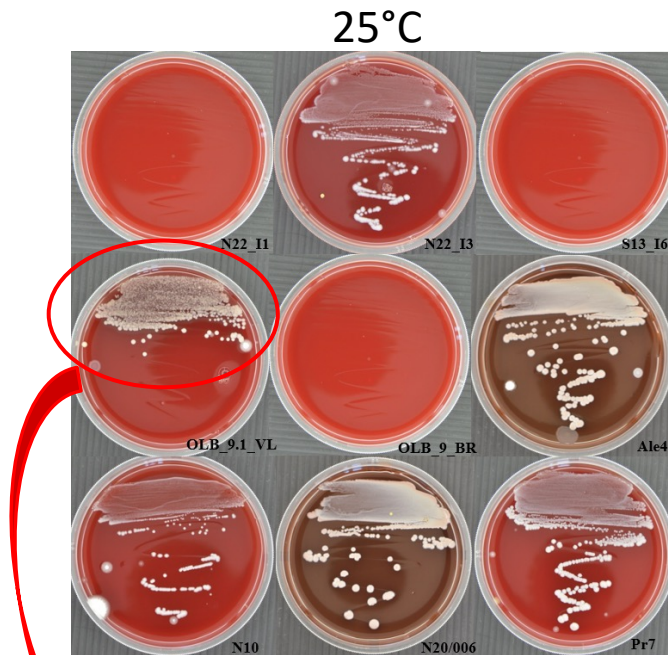
Yeast isolate	SF _L	SF _S	
		+4°C	+25°C
<i>M. pulcherrima</i> Ale 4	1.00±0.004	0.89±0.003	0.00±0.0
<i>M. purcherrima</i> N20/006	1.00±0.001	0.77±0.001	0.00±0.0
<i>M. pulcherrima</i> Pr7	1.00±0.004	0.80±0.004	0.67±0.002
<i>L. thermotholerans</i> N10	1.00±0.0	0.89±0.001	0.90±0.001
<i>S. bacillaris</i> N22_I1	1.00±0.0	0.85±0.01	0.90±0.02
<i>S. diversa</i> N22_I3	1.00±0.0	0.70±0.01	0.78±0.01
<i>A. pullulans</i> OLB_9_VL	1.00±0.0	0.67±0.003	0.47±0.003
<i>H. uvarum</i> OLB_9_BR	0.98±0.01	0.00±0.0	0.00±0.0
<i>S. bacillaris</i> S13_I6	1.00±0.0	0.78±0.002	0.00±0.0

Better yeast shelf-life at **4°C** for up to 30 days



**GOOD PERSPECTIVE FOR
TECHNOLOGICAL DEVELOPMENT**

- Evaluation of α and β hemolysis of the 9 yeasts, through growth on blood agar plates incubated at different temperatures for 14 days.
- Nutrient substrate with defibrinated sheep's blood, to evaluate the possible **hemolytic activity** of the microorganisms.



CONCLUSIONS

- 🍇 Collection of 36 non-*Saccharomyces* yeasts characterized at CREA-VE



9 selected as good BCAs against *B. cinerea*:

- ✓ 3 *M. pulcherrima*
- ✓ 1 *L. thermotolerans*
- ✓ 2 *S. bacillaris*
- ✓ 1 *S. diversa*
- ✓ 1 *H. uvarum*
- ✓ 1 *A. pullulans*

- 🍇 Defined mechanism of action for yeasts with antibotrytic activity:

- ❖ **Production of diffusible substances** (*S. bacillaris* N22_I1, *S. diversa*, *L. thermotolerans*, *M. pulcherrima* Pr7)
- ❖ **Production of volatile organic compounds** (*S. diversa*, *A. pullulans*, *M. pulcherrima* Ale4 and N20/006)
- ❖ **Competition for space and nutrients** (*S. bacillaris* S13_I6, *H. uvarum*)

- 🍇 Verified excellent adaptability to technological development

PERSPECTIVES

- 🍇 Expansion of the microbiological collection
- 🍇 Evaluation of antibotrytis efficacy in open field (ongoing)
- 🍇 Influence of yeasts in mixed vinifications



THANK YOU FOR YOUR ATTENTION