

Microbes vs Microbes

Controlling production of Aflatoxin in post-harvest handling by adding antagonistic microorganisms

case : coffee post-harvest

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Webinar – New approach to control Aflatoxin production during post-harvest handling in spices
28th of June, 2023

Outline

01

Introduction

Microbe and Us

02

War of Microbe

Glimpse of microbial antagonistic study

03

Anti-mold

Our case with coffee

Studying Microbe

Study-Perspective on Microbe

1650 - 1700 - 1750 - 1800

1677 - Leeuwenhoek melihat mikroorganisme

1796 - E. Jenner menemukan vaksin cacar

1850an

1850 - Semmelweis advokasi cuci tangan

1859 - Darwin, 'On the Origin of Species'

Pasteur - membantah 'spontaneous generation'
Pasteur - support germ theory of disease

1860an
Leeuwenhoek - mikroba dari gigi
Lister - antiseptic surgery

1876 - Koch membuktikan teori 'Germ' (anthraks)

1880an
Koch - menumbuhkan bakteri pada media padat
Ehrlich & Gram - mewarnai mikroba
Pasteur - vaksinasi rabies
Petri - penemuan cawan petri

Metchinkoff - istilah mikrobiome

1890an
Ivanovski - menemukan virus
Buchner - penemuan enzim, fermentasi tanpa mikroba

1900an

1928 - Fleming menemukan penisilin

1940an - Fermentasi skala industri, produksi masal antibiotik

1950an

1944 - DNA sebagai materi genetik

1953 - struktur DNA

1970an
1977, Woese, domain of life
1977, Sequencing, membaca DNA
1978, menggandakan DNA dengan PCR

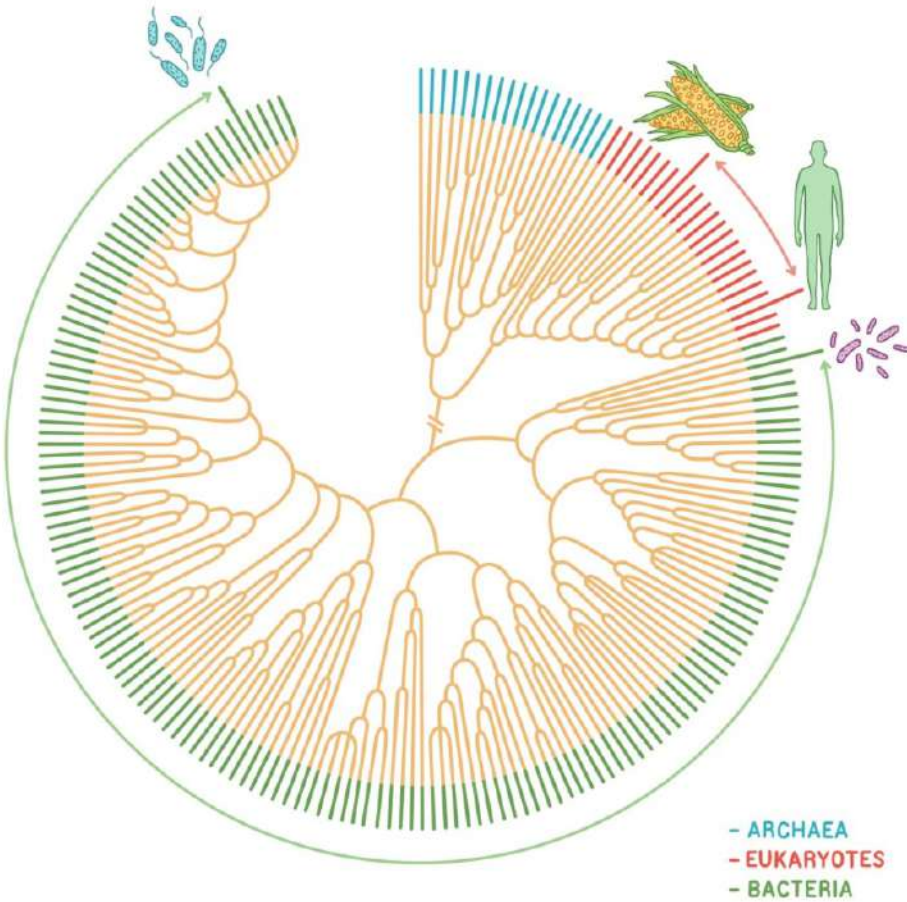
1990an
1990, Human Genome Project dimulai
1995, Urutan lengkap genom mikroorganisme pertama, H. influenzae
1998, Pyrosequencing

2000an

2000an
2001, Human Genome Project selesai
2005, 2nd Generation Sequencing (NGS)
2007, Metagenomic - diversitas mikroba
2008, 3rd Generation Sequencing (Nanopore)
2010, \$1000 genome

2010an
2014, DNA-editing, CRISPR-Cas9
2012-17
Earth Microbiome Project
American Gut Project
International Microbiome Initiative
Integrative Human Microbiome Project
2019, Portable sequencing (MinION, Nanopore)

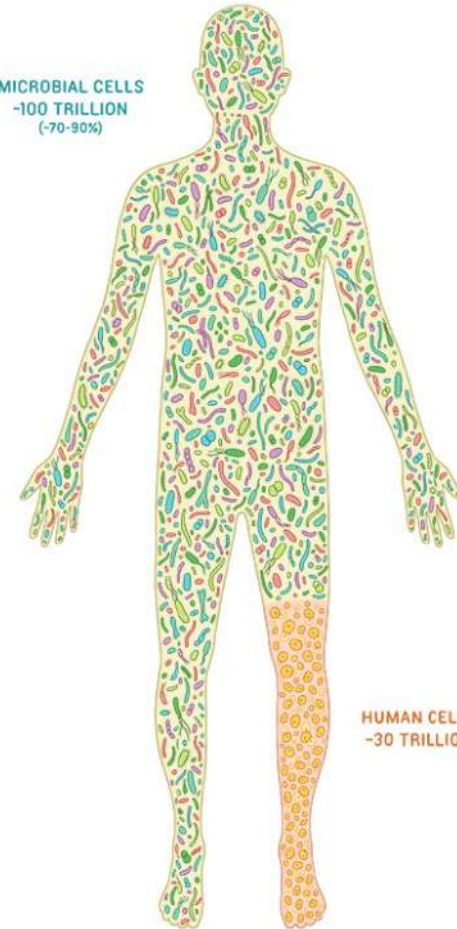
Microbes & Us



On the tree of life, humans are far closer to corn, a green plant, than two common bacteria are to each other.

by Gaby D'Alessandro / © AMNH

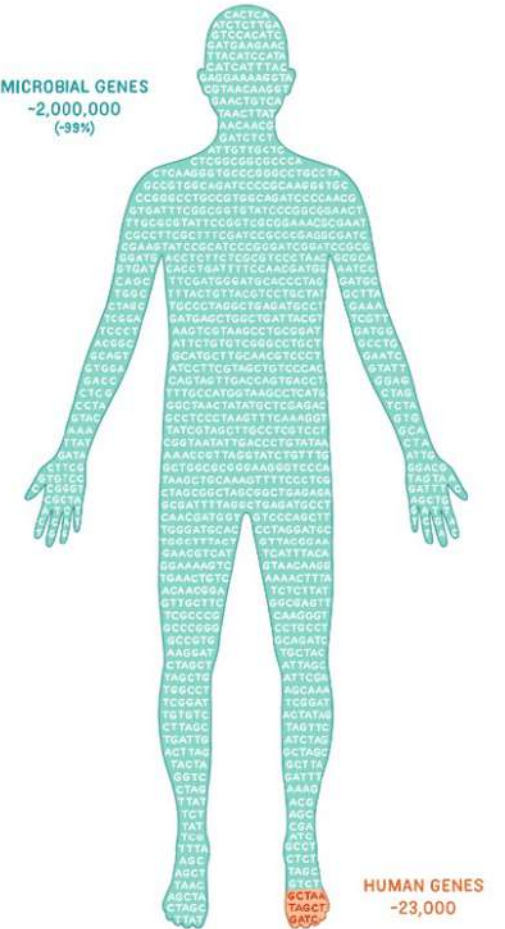
MICROBIAL CELLS
~100 TRILLION
(~70-90%)



An estimated 30 trillion cells in your body—less than a third—are human. The other 70-90% are bacterial and fungal.

by Gaby D'Allesandro / © AMNH

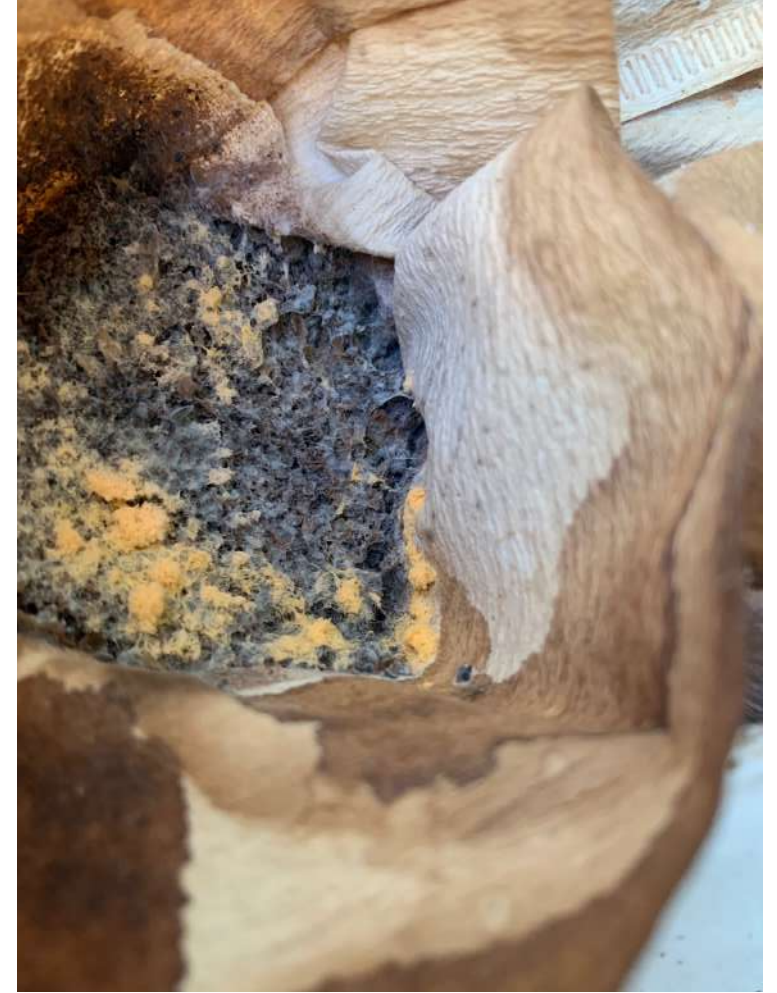
MICROBIAL GENES
~2,000,000
(~99%)



Ninety-nine percent of the unique genes in your body are bacterial. Only about one percent is human.

by Gaby D'Allesandro / © AMNH

Microbes is all around



Study on 'War of Microbes'

MYCOBIOLOGY, 2018
VOL. 46, NO. 1, 33–46
<https://doi.org/10.1080/12298093.2018.1454013>



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RESEARCH ARTICLE

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Screening and Evaluation of Yeast Antagonists for Biological Control of *Botrytis cinerea* on Strawberry Fruits

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ABSTRACT

Gray mold (*Botrytis cinerea*) is one of the most common diseases of strawberries (*Fragaria × ananassa* Duchesne) worldwide. Although many chemical fungicides are used for controlling the growth of *B. cinerea*, the risk of the fungus developing chemical resistance together with consumer demand for reducing the use of chemical fungicides have necessitated an alternative method to control this pathogen. Various naturally occurring microbes aggressively attack plant pathogens and benefit plants by suppressing diseases; these microbes are referred to as *biocontrol agents*. However, screening of potent biocontrol agents is essential for their further development and commercialization. In this study, 24 strains of yeast with antagonistic ability against gray mold were isolated, and the antifungal activity of the volatile and diffusible metabolites was evaluated. Putative mechanisms of action associated with the biocontrol capacity of yeast strains against *B. cinerea* were studied through *in vitro* and *in vivo* assays. The volatile organic compounds produced by the *Galactomyces candidum* JYC1146 could be useful in the biological control of plant pathogens and therefore are potential alternative fungicides with low environmental impact.



Figure 4. Efficacy of the volatiles produced by antagonistic yeast in postharvest control of *B. cinerea* on strawberry. Yeast cultures and strawberries with *B. cinerea* spores on the surface were placed into a sealed plastic box at high relative humidity to ensure favorable conditions for the postharvest onset of the disease.

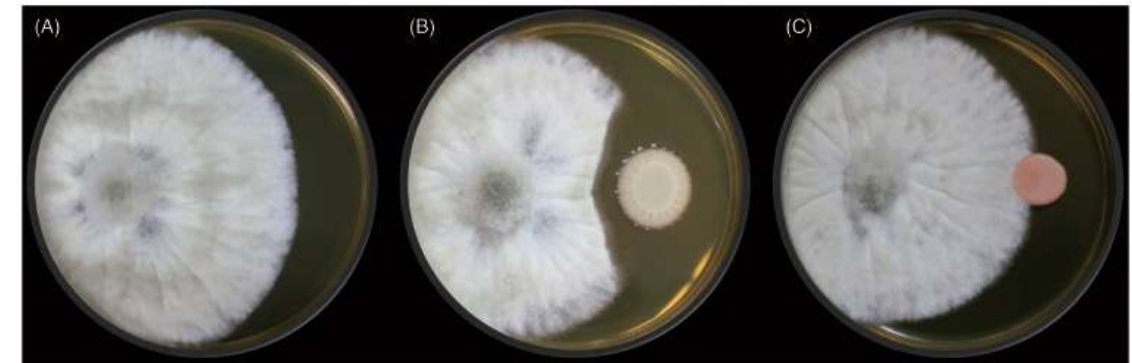


Figure 5. Evaluation of *in vitro* antagonism by production of diffusible substances by yeasts against the *B. cinerea* JYC2142 on YPD plate. (A) *B. cinerea* alone as the control group. (B) Yeasts (right) were antagonistic to *B. cinerea* (left). (C) Yeasts (right) were not antagonistic to *B. cinerea* (left).

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www.elsevier.com/locate/ijfoodmicro

Short Communication

The effects of yeasts involved in the fermentation of *Coffea arabica* in East Africa on growth and ochratoxin A (OTA) production by *Aspergillus ochraceus*

Wafa Masoud *, Christa Høj Kaltoft

Department of Food Science, Food Microbiology, The Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

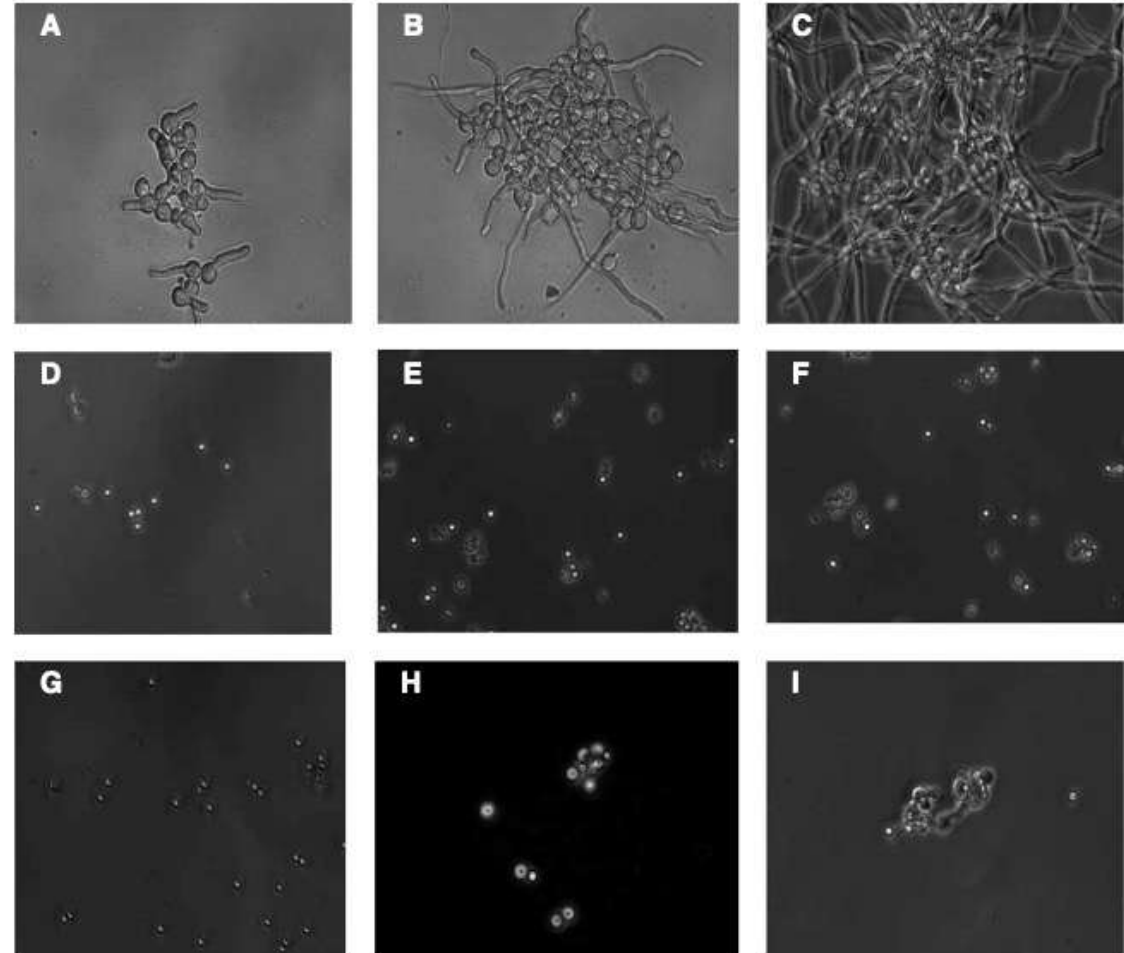


Fig. 2. Germination of *A. ochraceus* B722 spores incubated at 30 °C when inoculated in MYGP broth without yeast after 24 h (A), 48 h (B) and 72 h (C); when co-cultured with cells of *P. anomala* S12 in MYGP broth after 24 h (D), 48 h (E) and 72 h (F); when inoculated in MYGP yeast-free supernatant after 24 h (G), 48 h (H), 48 h (I) and 72 h (J).

Study on ‘War of Microbes’

Yeast

Yeast 2005; 22: 1133–1142.

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Research Article

Influence of volatile compounds produced by yeasts predominant during processing of *Coffea arabica* in East Africa on growth and ochratoxin A (OTA) production by *Aspergillus ochraceus*

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Abstract

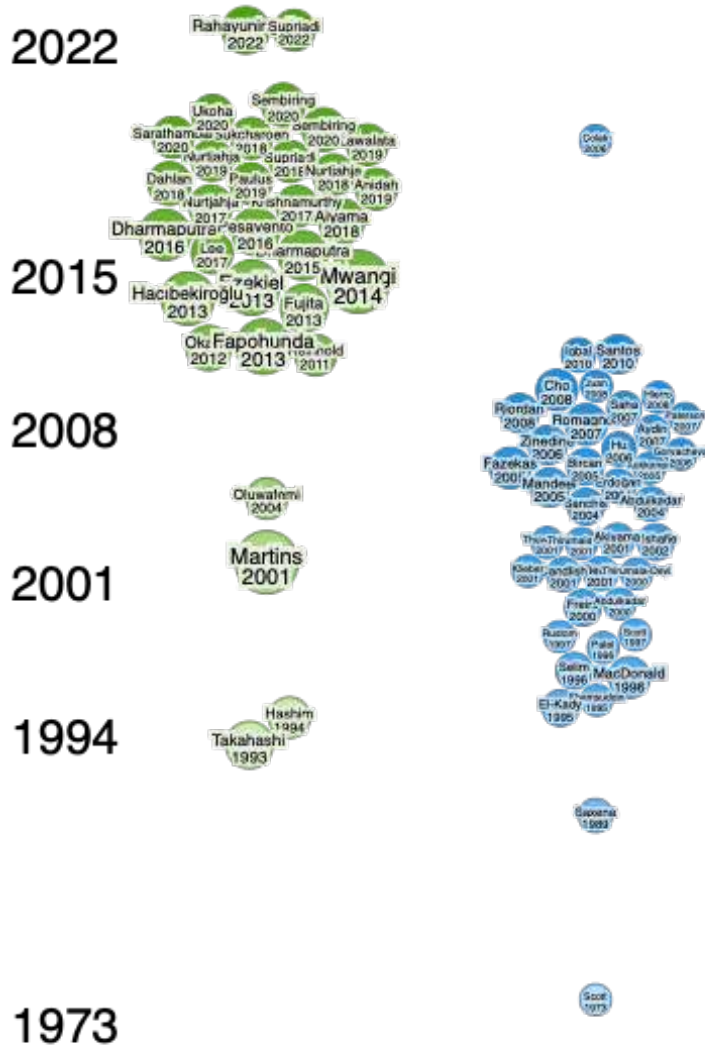
The effects of volatile compounds produced during coffee processing by *Pichia anomala*, *P. kluyveri* and *Hanseniaspora uvarum* on growth of *Aspergillus ochraceus* and production of ochratoxin A (OTA) were studied. On malt extract agar (MEA) and on coffee agar (CA), exposure of *A. ochraceus* to the gaseous phase of malt yeast glucose peptone (MYGP) plates inoculated with *P. anomala*, *P. kluyveri* and *H. uvarum* inhibited fungal growth, with the two *Pichia* spp. showing the strongest effect. The main esters and alcohols produced by the three yeasts were ethyl acetate, isobutyl acetate, 2-phenyl ethyl acetate, ethyl propionate and isoamyl alcohol. The individual esters and alcohols were found to affect fungal growth. The most effective compound in inhibiting fungal growth was 2-phenyl ethyl acetate; which at 48 µg/l headspace completely inhibited growth of *A. ochraceus*. Exposure of *A. ochraceus* to the gaseous phase of MYGP plates inoculated with *P. anomala*, *P. kluyveri* and *H. uvarum* prevented production of OTA. On CA medium, only the headspace of *P. anomala* and *P. kluyveri* prevented OTA production. Furthermore, when *A. ochraceus* was exposed to the headspace of the individual volatile compounds, 2-phenyl ethyl acetate was the most effective in preventing OTA production. Prevention of OTA seems to be due to reduction of fungal biomass. Copyright © 2005 John Wiley & Sons, Ltd.

Keywords: coffee; *Pichia anomala*; *Pichia kluyveri*; *Hanseniaspora uvarum*; *Aspergillus ochraceus*; OTA; volatile compounds

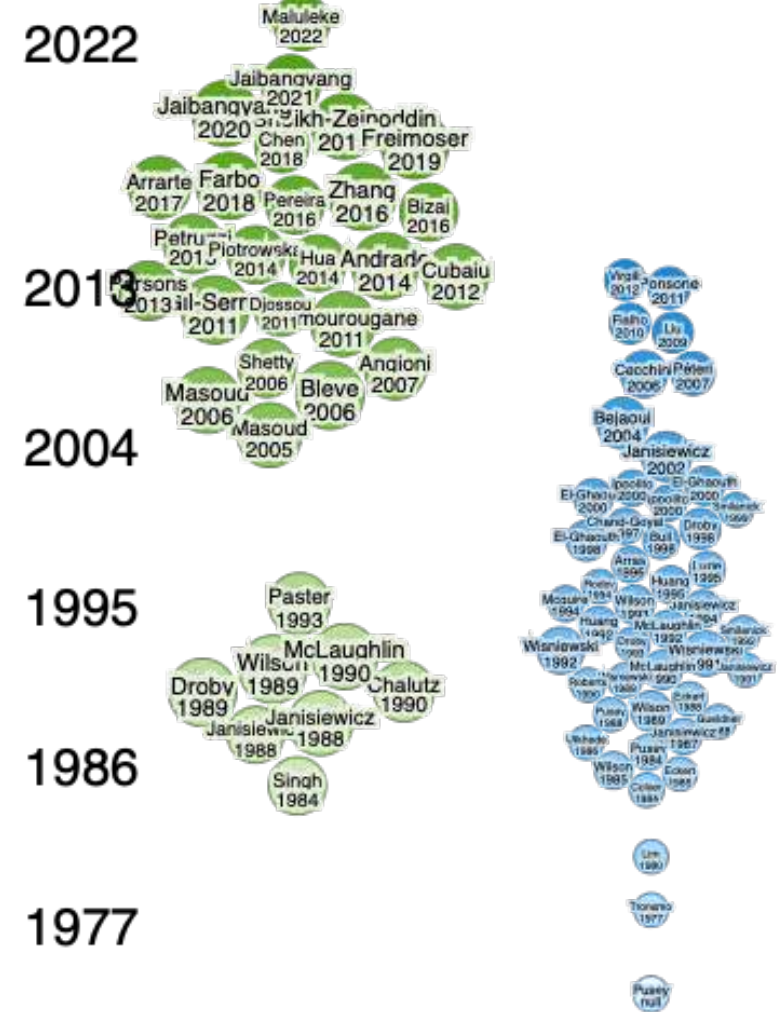
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Study on 'War of Microbes'

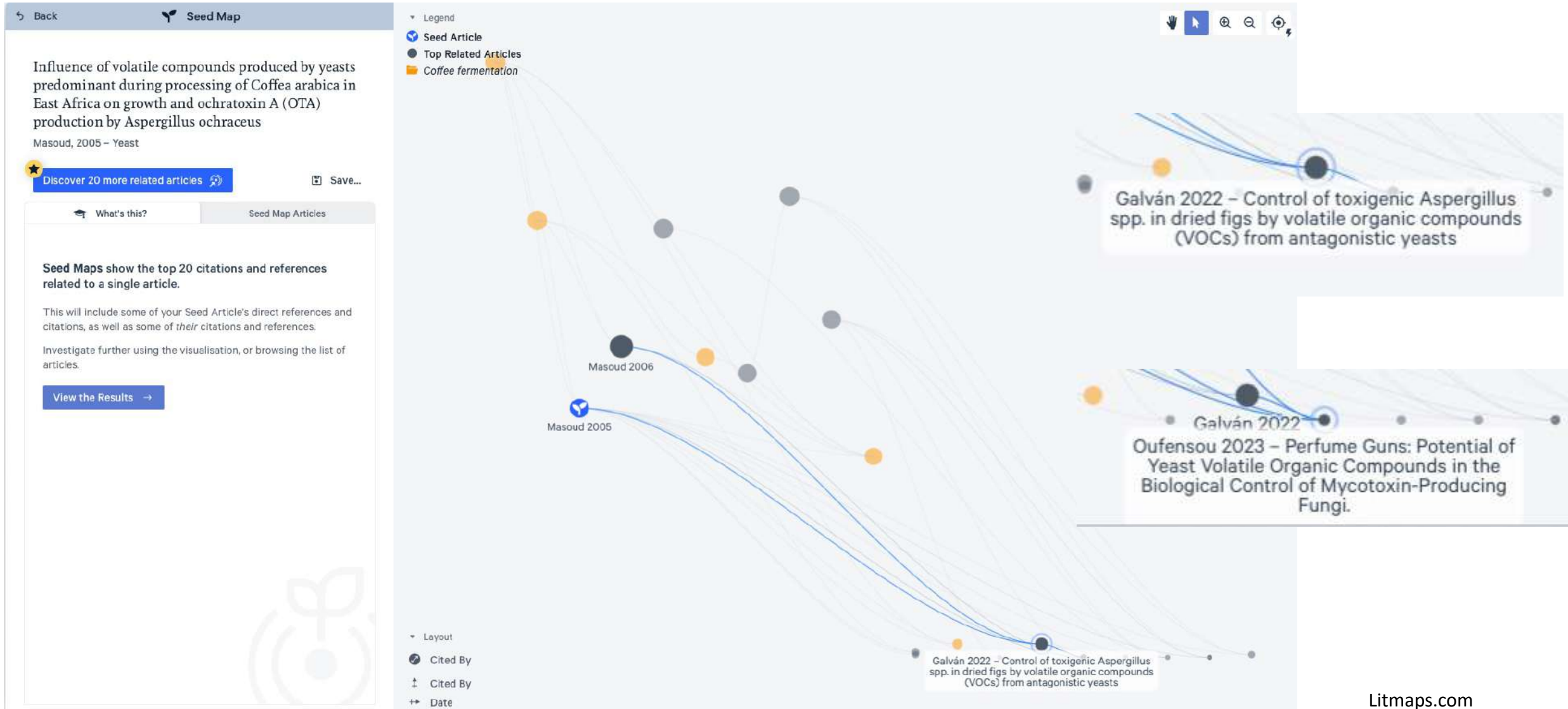
Aflatoxin & Nutmeg



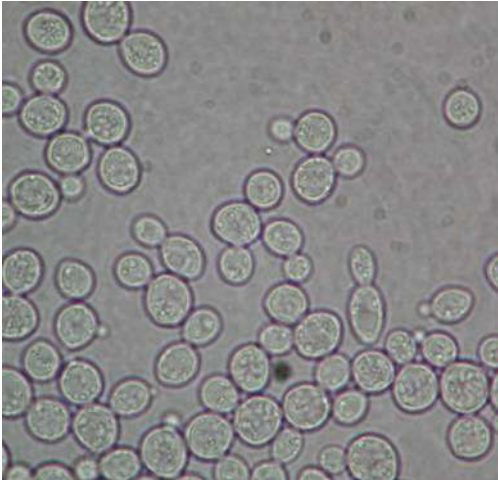
Yeast fighting *Aspergillus*+



Study on 'War of Microbes'



Our Research : coffee post-harvest

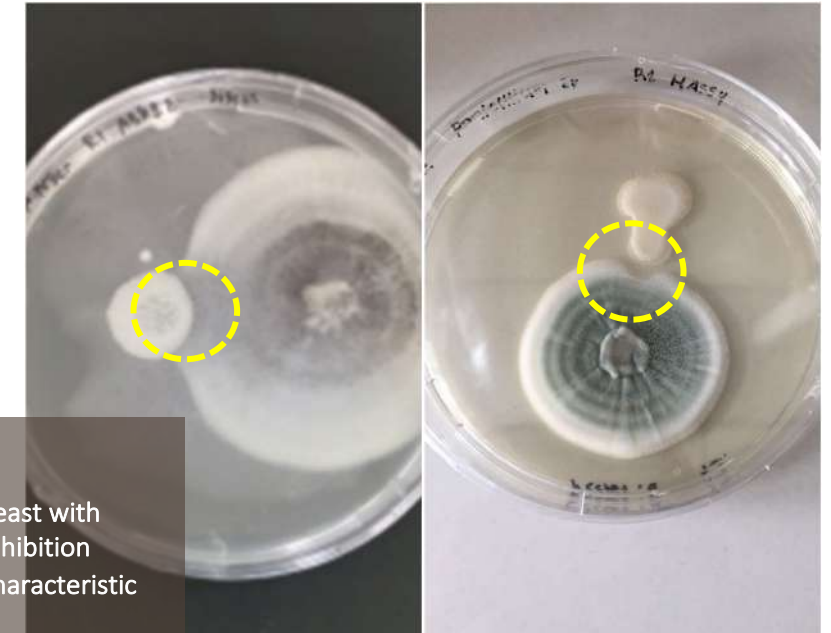


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- *Screening 73* yeast isolates
- **46** potensial isolates



Yeast without
inhibition
characteristics



Yeast with
inhibition
characteristic

Collaboration with PT Singa Garuda Mas (MF Program)

Field trial : coffee post-harvest in Bandung



Without anti-mold

- 'white spots/stripes' (indication of filamentous fungi)
- *Cupping score: 78,75*

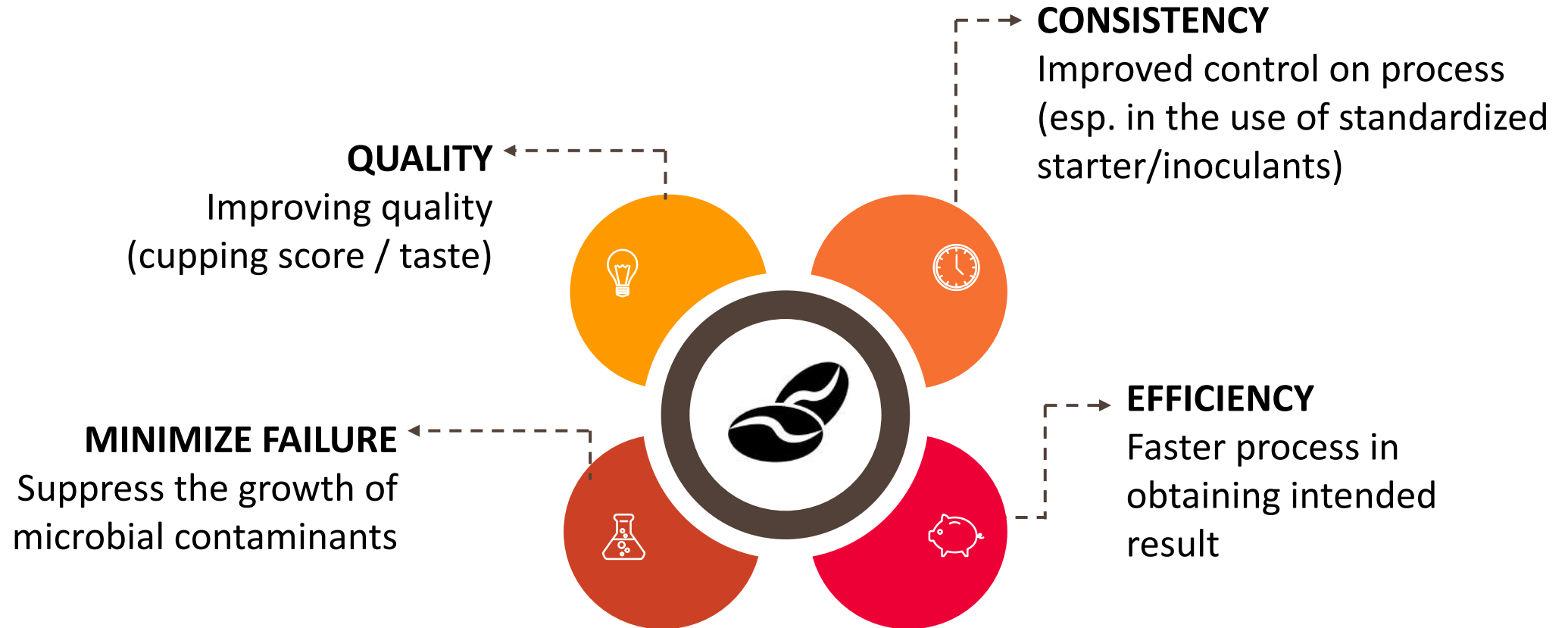
With antimold

- No 'white spots/stripes'
- *Cupping score: 84,5*



Winning in 'War of Microbes'

*case in coffee post-harvest





Thank you