

# Fungos ocratoxigênicos e ocratoxina A em solos, folhas, uvas e vinhos do Vale do São Francisco



**Prof Luís Roberto Batista - UFLA**

**SEMINÁRIO**

**Indicação Geográfica para os Vinhos Finos do Vale do São Francisco - Petrolina  
17 e 18 de outubro de 2017**

**Local: Auditório do Senac, Petrolina-PE**

# Micotoxinas em Alimentos



☞ Micotoxinas em alimentos:

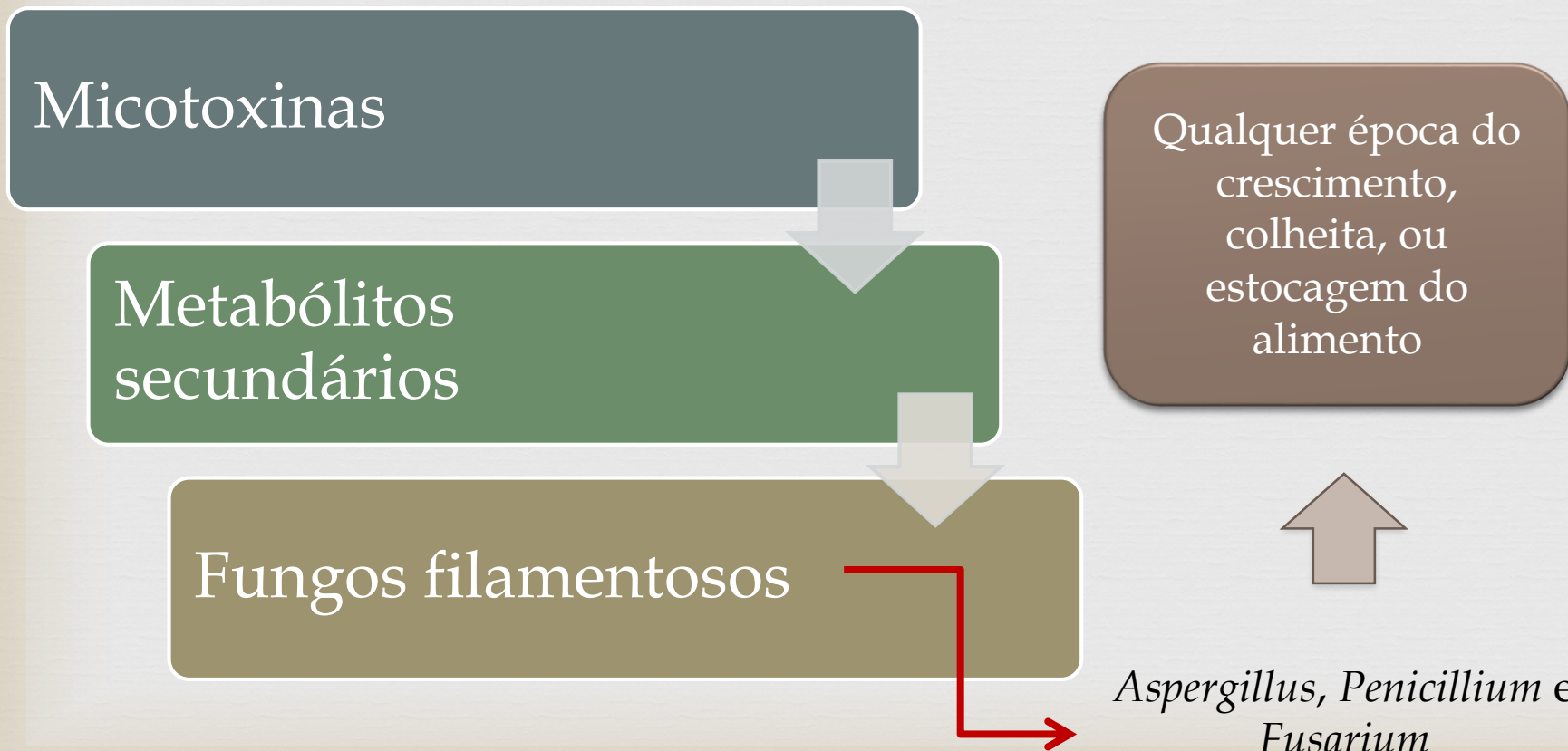
Micotoxinas

Metabólitos secundários

Fungos filamentosos

Qualquer época do crescimento, colheita, ou estocagem do alimento

*Aspergillus, Penicillium e Fusarium*



# Micotoxinas em Alimentos



OTA – Micotoxina  
*Aspergillus* e  
*Penicillium*



Cereais  
Amido



Café, cacau,  
cerveja, uva e  
derivados, entre  
outros.



Europa e Brasil

2 µg/L



# Risco de Ocratoxina A em vinhos



Produção do vinho tinto



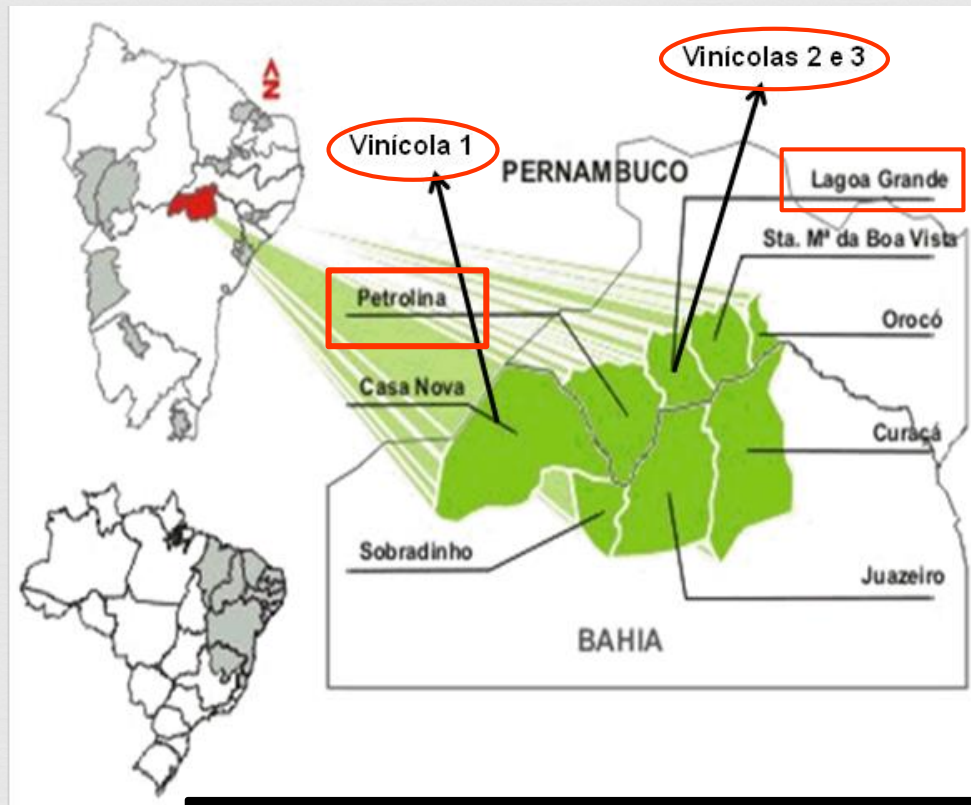
# Produção de Ocratoxina A por fungos do gênero *Aspergillus*



OTA  
Resveratrol

- Temperatura;
- Localização;
- $A_w$ ;
- Luz UV;
- Processo de vinificação;
- Infestação fúngica;
- Solo de cultivo.

# ÁREA DE ESTUDO



Latitude

9°S

Longitude

40°W

Temperatura média anual (°C)

27

Precipitação anual (mm)

500

Tipo de clima

Tropical Semi-Árido



# Números do projeto (2013-2017)



| Números do projeto (2013-2017)               |      |
|--|------|
| Estudante de Pós-doutorado (2015-2016)       | 1    |
| Estudante de Iniciação Científica            | 7    |
| Dissertações de Mestrado                     | 3    |
| Tese de Doutorado                            | 2    |
| Resumos em Eventos Científicos Nacionais     | 15   |
| Rsumos em Eventos Científicos Internacionais | 5    |
| Artigos Científicos Em Elaboração            | 3    |
| Artigos Científicos Publicados               | 4    |
| Número de fungos isolados e identificados    | 2356 |
| Números de fungos preservados                | 210  |
| Número de Amostras Analisadas                | 120  |

# RECENTES RESULTADOS APRESENTADOS EM EVENTOS CIÊNTÍFICOS



☞ FREIRE, L.; PASSAMANI, F. R. F. ; FREIRE, C. A. ; SCHABO, D. C. ; BATISTA, L. R. . INFLUÊNCIA DE FATORES ABIÓTICOS NA PRODUÇÃO DE OCHRATOXINA A POR ASPERGILLUS CARBONARIUS. In: VII CONGRESSO LATINO-AMERICANO E XII CONGRESSO BRASILEIRO DE HIGIENISTAS DE ALIMENTOS, 2015, BÚZIOS. HIGIENE ALIMENTAR, 2015.

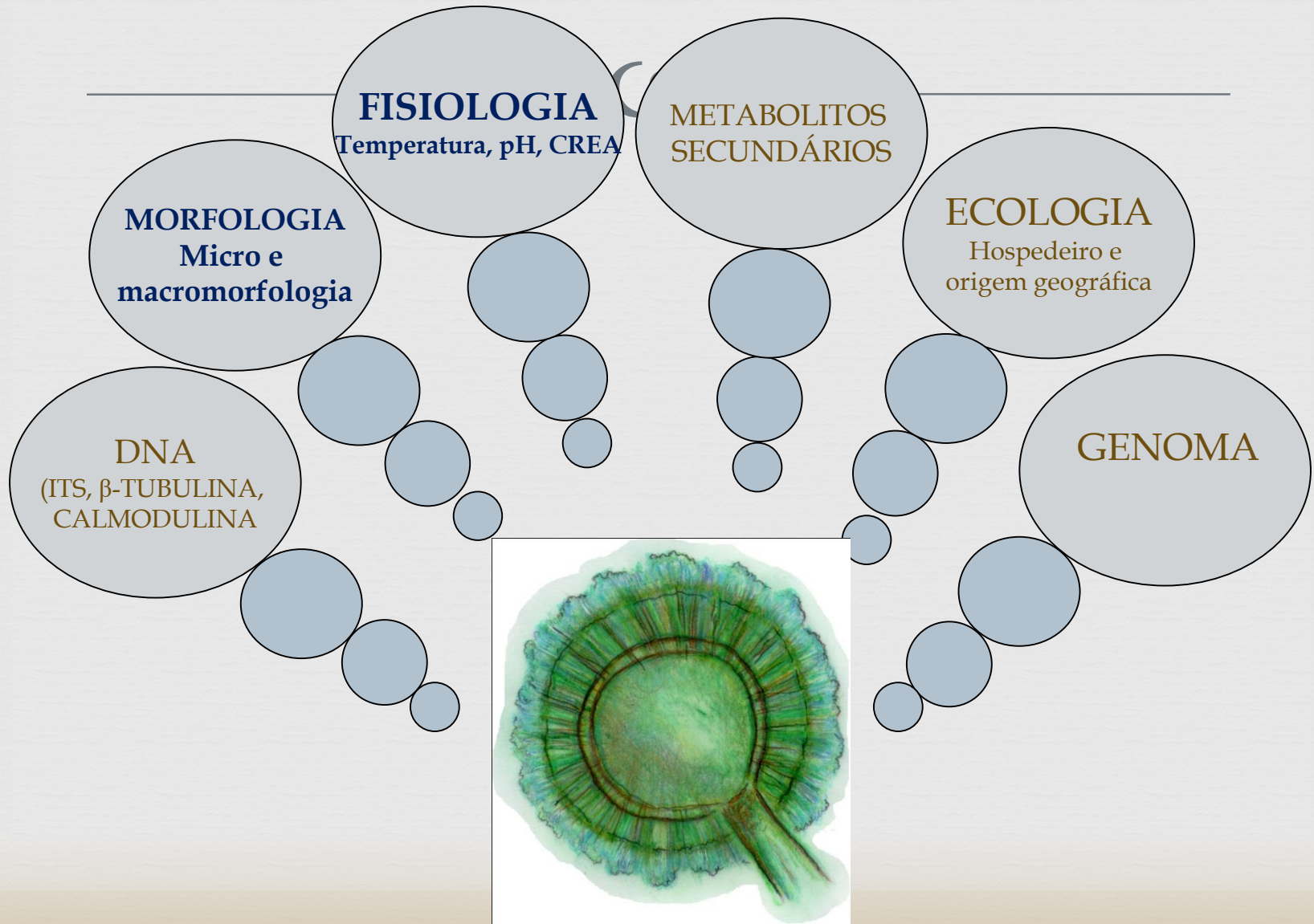
☞ FREIRE, l.; PASSAMANI, F. R. F., PRADO, G.; BATISTA, L. R. INFLUÊNCIA DAS VARIEDADES DE UVAS VINÍFERAS E DA SAFRA NA INCIDÊNCIA FÚNGICA. In: Congresso Latino-Americano de Micologia, Florianópolis, Brasil, 2016.

☞ FREIRE, l.; PASSAMANI, F. R. F., PEREIRA, G. E.; BATISTA, L. R.. DIVERSIDADE DE ESPÉCIES DE ASPERGILLUS E PENICILLIUM EM SOLOS DE VINHEDOS DA REGIÃO TROPICAL SEMIÁRIDA DO BRASIL. In: Congresso Latino-Americano de Micologia, Florianópolis, Brasil, 2016.

☞ FREIRE, LUÍSA; PASSAMANI, F. R. F.; Thomas, A. B.; NASSUR, R. C. M. R.; SILVA, L. M.; PASCHOAL, F. N.; Pereira, G. E.; PRADO, G.; BATISTA, L. R.. Influence of physical and chemical characteristics of wine grapes on the incidence of Penicillium and Aspergillus fungi in grapes and ochratoxin A in wines. In: 7Th Congress of European Microbiologists, 2017, Valência-Espanha. 7Th Congress of European Microbiologists. 2017.



# Taxonomia Polifásica



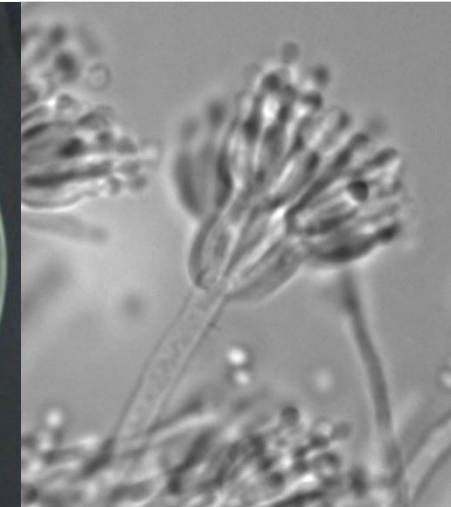
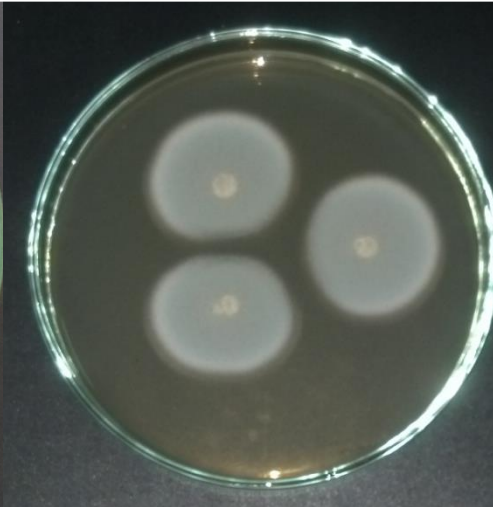
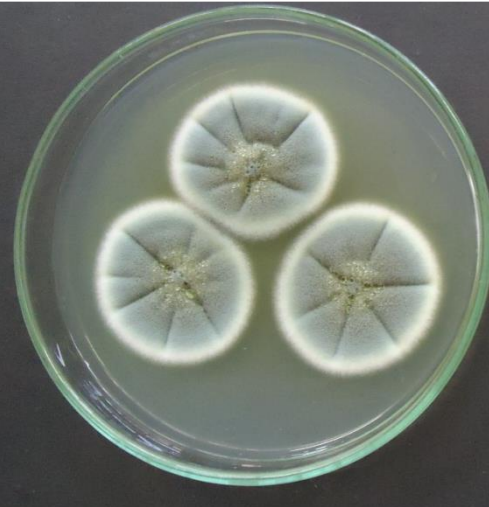
# Taxonomia Polifásica

CYA 25

MEA 25

CYA 37

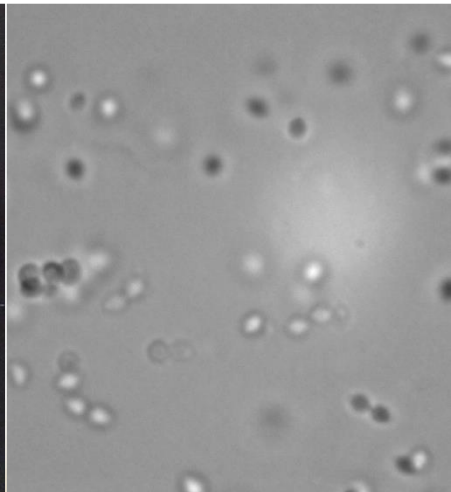
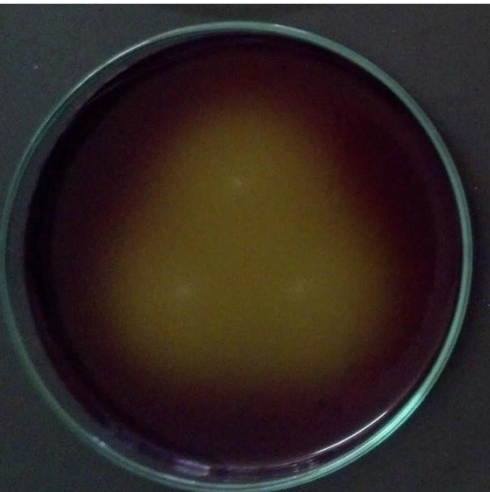
Microcopia



CYA 30

YES 25

CREA 25





# Espécies do gênero *Aspergillus* Seção *Nigri*



*A. carbonarius*



*A. niger*



*A. tubingensis*



*A. japonicus*



*A. aculeatus*



# VARIEDADES ESTUDADAS



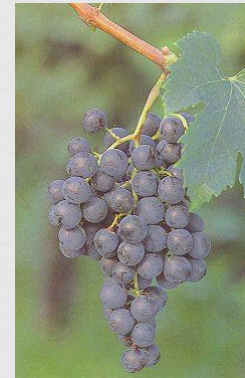
Syrah



Petit Verdot



Cabernet Sauvignon



Merlot



Chenin Blanc

Moscato canelli

Tempranillo

# Influence of physical and chemical characteristics of wine grapes on the incidence of *Penicillium* and *Aspergillus* fungi in grapes and ochratoxin A in wines

Luísa Freire <sup>a</sup>, Fabiana Reinis Franca Passamani <sup>a</sup>, Ariela Betsy Thomas <sup>a</sup>, Rita de Cássia Mirela Resende Nassur <sup>b</sup>, Lais Mesquita Silva <sup>a</sup>, Fabiano Narciso Paschoal <sup>c</sup>, Giuliano Elias Pereira <sup>b</sup>, Guilherme Prado <sup>c</sup>, Luís Roberto Batista <sup>a</sup> ✉

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<https://doi.org/10.1016/j.ijfoodmicro.2016.10.027>

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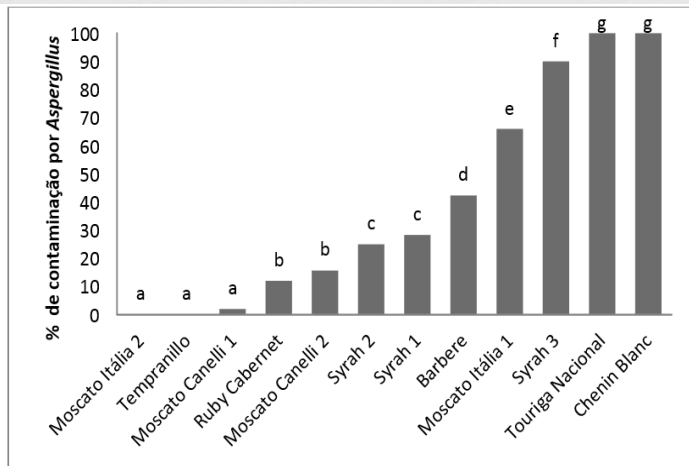


Gráfico 1 – Percentual de contaminação de uvas viníferas por fungos do gênero *Aspergillus* e as respectivas proporções médias de colonização. Letras diferentes mostram diferença estatística significativa a  $p < 0,05$ .

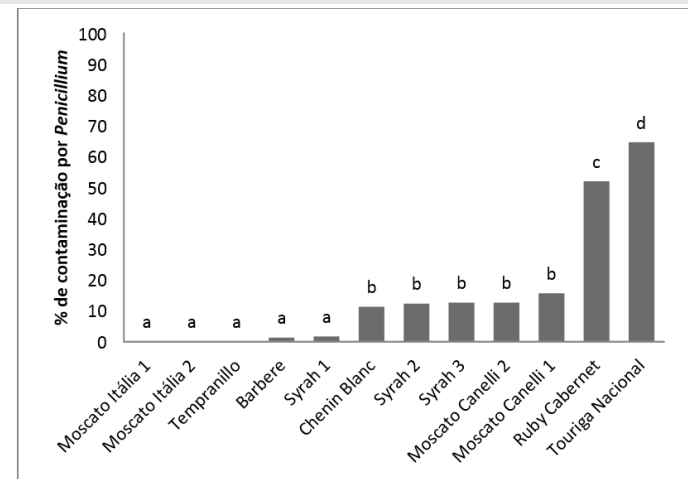


Gráfico 2 – Percentual de contaminação de uvas viníferas por fungos do gênero *Penicillium* e as respectivas proporções médias de colonização. Letras diferentes mostram diferença estatística significativa a  $p < 0,05$ .

View issue TOC  
Volume 93, Issue 4  
15 March 2013  
Pages 890-894

## Research Article

# Detection of ochratoxin A in tropical wine and grape juice from Brazil

Michelle F Terra, Guilherme Prado, Giuliano E Pereira, Hugo J Ematné, Luís R Batista ✉

First published: 27 July 2012 Full publication history

DOI: 10.1002/jsfa.5817 View/save citation

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## Abstract

**BACKGROUND:** Ochratoxin A (OTA) is the main mycotoxin found in grapes, wines and grape juices and is considered one of the most harmful contaminants to human health. In this study, samples of tropical wines and grape juices from different grape varieties grown in Brazil were analysed for their OTA content by high-performance liquid chromatography.

**RESULTS:** The detection and quantification limits for OTA were 0.01 and 0.03  $\mu\text{g L}^{-1}$  respectively. OTA was detected in 13 (38.24%) of the samples analysed, with concentrations ranging from < 0.03 to 0.62  $\mu\text{g L}^{-1}$ . OTA was not detected in any of the grape juice samples. Most of the red wine samples proved to be contaminated with OTA (75%), while only one white wine sample was contaminated. However, the OTA levels detected in all samples were well below the maximum tolerable limit (2  $\mu\text{g L}^{-1}$ ) in wine and grape juice established by the

[Home](#) > [Journal of Food Protection](#) > [September 2016](#) > Effect of Fungicides on Growth and Ochratoxin A Production by Aspergil...[Advanced Search](#)Volume 79, Issue 9  
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### Article Citation:

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### Research Papers

#### Effect of Fungicides on Growth and Ochratoxin A Production by *Aspergillus carbonarius* from Brazilian Wine Grapes

MICHELLE F. TERRA,<sup>1</sup> NATHASHA de A. LIRA,<sup>1</sup> FABIANA R. F. PASSAMANI,<sup>1</sup> WILDER DOUGLAS SANTIAGO,<sup>2</sup> MARIA das GRAÇAS CARDOSO,<sup>2</sup> LUÍS ROBERTO BATISTA<sup>1\*</sup><sup>1</sup>Department of Food Science, Federal University of Lavras, Lavras, Minas Gerais, 37200-000, Brazil<sup>2</sup>Department of Chemistry, Federal University of Lavras, Lavras, Minas Gerais, 37200-000, Brazil\* Author for correspondence. Tel and Fax: +55 3538291407; E-mail: [luisrb@dca.ufla.br](mailto:luisrb@dca.ufla.br).

### ABSTRACT

Prevention in the field of mycotoxin-producing fungi is the most effective strategy for controlling the presence of mycotoxins in foods. Chemical fungicides are widely used to protect crops, so their implications on mycotoxin production need to be considered. Therefore, the aim of this study was to evaluate the effect in vitro and on grapes of five fungicides commonly used

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## Effect of Temperature, Water Activity, and pH on Growth and Production of Ochratoxin A by *Aspergillus niger* and *Aspergillus carbonarius* from Brazilian Grapes

FABIANA REINIS FRANCA PASSAMANI,<sup>1\*</sup> THAIS HERNANDES,<sup>2</sup> NOELLY ALVES LOPES,<sup>3</sup>  
SABRINA CARVALHO BASTOS,<sup>1</sup> WILDER DOUGLAS SANTIAGO,<sup>4</sup> MARIA DAS GRAÇAS CARDOSO,<sup>4</sup>  
AND LUÍS ROBERTO BATISTA<sup>1</sup>

<sup>1</sup>Department of Food Science, <sup>3</sup>Department of Biology, and <sup>4</sup>Department of Chemistry, Federal University of Lavras, Lavras, Minas Gerais, 37200-000, Brazil; and <sup>2</sup>School of Nutrition, Federal University of Mato Grosso, Cuiabá, Mato Grosso, 78060-900, Brazil

MS 13-495: Received 18 November 2013/Accepted 24 June 2014

### ABSTRACT

The growth of ochratoxigenic fungus and the presence of ochratoxin A (OTA) in grapes and their derivatives can be caused by a wide range of physical, chemical, and biological factors. The determination of interactions between these factors and fungal species from different climatic regions is important in designing models for minimizing the risk of OTA in wine and grape juice. This study evaluated the influence of temperature, water activity ( $a_w$ ), and pH on the development and production of OTA in a semisynthetic grape culture medium by *Aspergillus carbonarius* and *Aspergillus niger* strains. To analyze the growth conditions and production of OTA, an experimental design was conducted using response surface methodology as a tool to assess the effects of these abiotic variables on fungal behavior. *A. carbonarius* showed the highest growth at temperatures from 20 to 33°C,  $a_w$  between 0.95 and 0.98, and pH levels between 5 and 6.5. Similarly, for *A. niger*, temperatures between 24 and 37°C,  $a_w$  greater than 0.95, and pH levels between 4 and 6.5 were optimal. The greatest toxin concentrations for *A. carbonarius* and *A. niger* (10 µg/g and 7.0 µg/g, respectively) were found at 15°C,  $a_w$  0.99, and pH 5.35. The lowest pH was found to contribute to greater OTA production. These results show that the evaluated fungi are able to grow and produce OTA in a wide range of temperature,  $a_w$ , and pH. However, the optimal conditions for toxin production are generally different from those optimal for fungal growth. The knowledge of optimal conditions for fungal growth and production of OTA, and of the stages of cultivation in which these conditions are optimal, allows a more precise assessment of the potential risk to health from consumption of products derived from grapes.

In Brazil, the cultivation of wine grapes is increasingly important, with several regions of the country, with different climates, involved in this activity. The sub-medium region of the São Francisco Valley, northeastern Brazil, stands out as a pioneer in the production of grapes and wine under

Balkan endemic nephropathy, a disease found in the rural areas of Bulgaria, Romania, Serbia, Croatia, and Bosnia (17). *Aspergillus carbonarius* is one of the main species responsible for OTA contamination in grapes, with higher production of OTA than other members of *Aspergillus*

# AVALIAÇÃO DO CRESCIMENTO DE *Aspergillus carbonarius* EM DIFERENTES TEMPERATURAS E ATIVIDADES DE ÁGUA

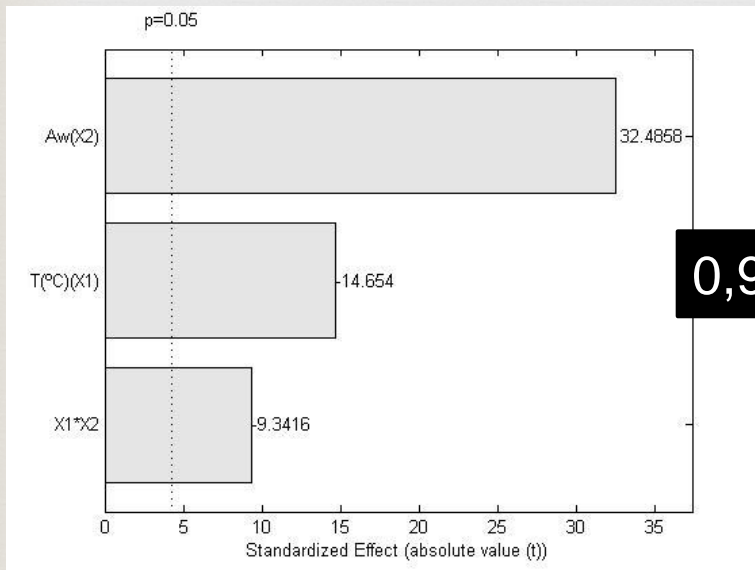
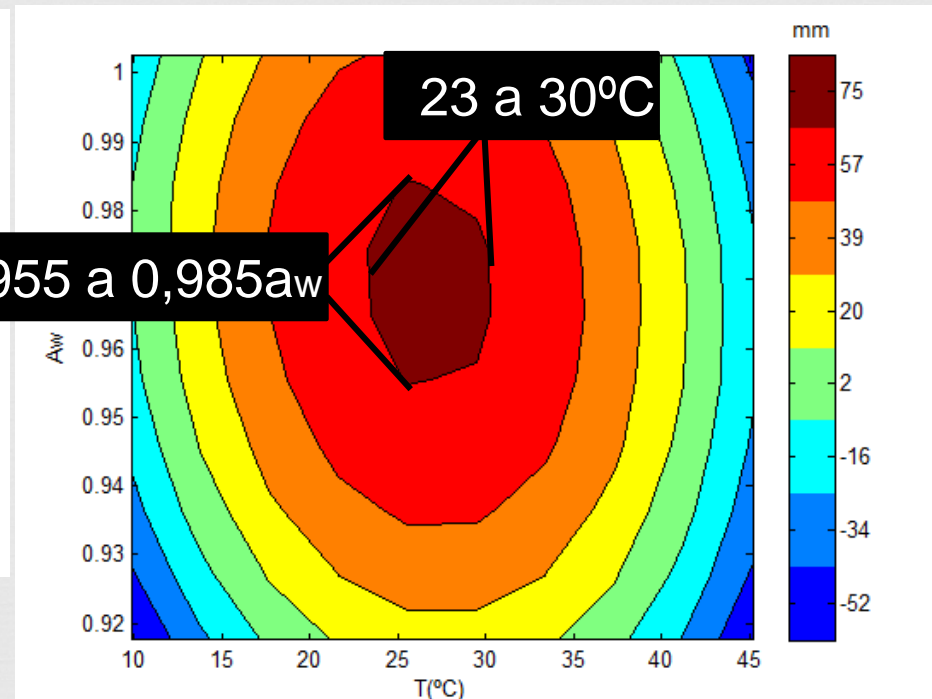


Diagrama de Pareto com efeito da temperatura (X1) e atividade de água (X2).



Curva de contorno para crescimento em função das interações entre temperatura e atividade de água

# AVALIAÇÃO DA PRODUÇÃO DE OTA POR *Aspergillus carbonarius* EM DIFERENTES TEMPERATURAS E ATIVIDADES DE ÁGUA

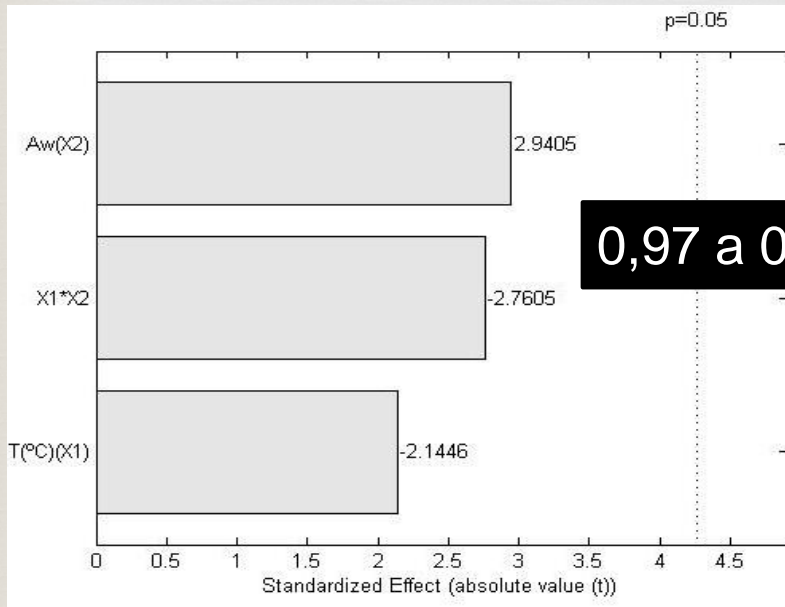
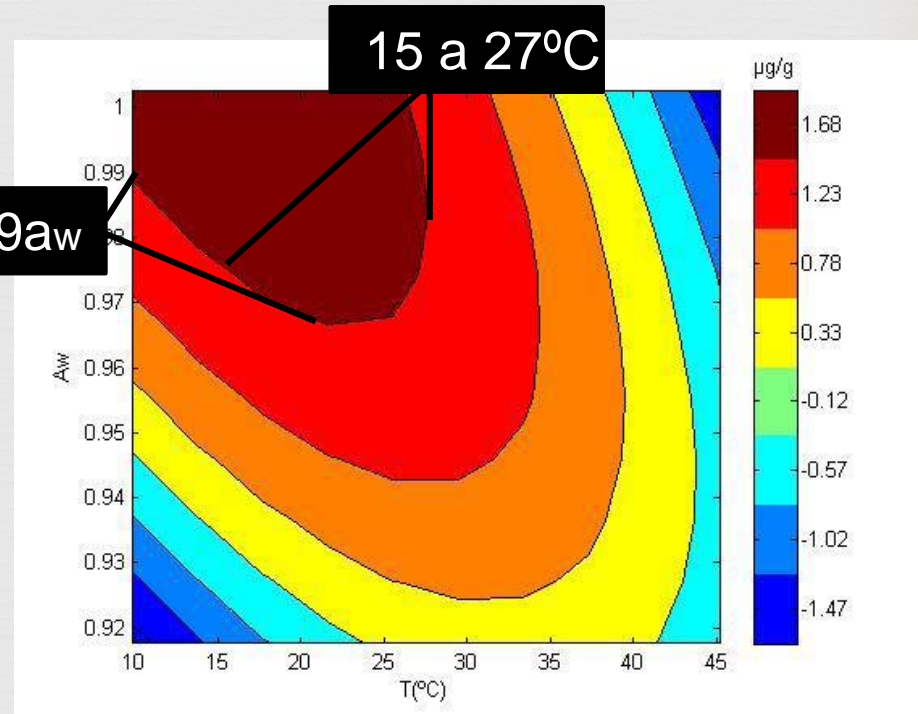


Diagrama de Pareto com efeito da temperatura (X1) e atividade de água(X2)



Curva de contorno para produção de OTA em função das interações entre temperatura e atividade de água



# Informações importantes



*Aspergillus  
carbonarius*



Maior produção de  
OTA nas uvas



Condições climáticas  
Atividade de água ( $a_w$ )  
Favoráveis

*A. carbonarius*

**Crescimento:**

T°: 20 °C a 33°C

Aw: 0,95 e 0,98

pH: 5 e 6,5

**Toxina - (10 µg/g)**

Aw: 0,99

T°: 15 °C

pH superior a 6,0

*A. niger*

**Crescimento:**

T°: 25 °C e 40 °C

Aw: superior a 0,96

pH: 4,0 e 6,5

**Toxina - (7 µg/g)**

Aw: 0,98

T°: 15 °C

# CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

## EDITAIS DE CREDENCIAMENTOS

O Secretário Executivo Substituto do Conselho de Gestão do Patrimônio Genético, em seu nome e no uso das competências que lhe foram conferidas pela Deliberação nº 203, de 19 de julho de 2007, com fundamento na Medida Provisória nº 2.186-16, de 23 de agosto de 2001, e no Decreto nº 3.945, de 28 de setembro de 2001, credencia como instituição fiel depositária de amostras de componente do patrimônio genético o Instituto de Estudos do Mar Almirante Paulo Moreira - IEAPM do Ministério da Marinha, nos seguintes termos:

|                                   |   |
|-----------------------------------|---|
| Nº do processo                    | 02000.001559/2015-77  |
| Instituição credenciada           | Instituto de Estudos do Mar Almirante Paulo Moreira - IEAPM do Ministério da Marinha                                  |
| CNPJ                              | 10.573.118/0001-62  |
| Coleção                           | Coleções IEAPM-PLAT; IEAPM-POR; IEAPM-ASC; IEAPM-CNID; IEAPM-MOL; IEAPM-ECHIN; IEAPM-BRYOZ; IEAPM-NEMAT e IEAPM-CRUST |
| Localidade das Coleções           | Arraial do Cabo/RJ  |
| Data da decisão de credenciamento | 13/11/2015  |
| Nº do credenciamento              | 142/2015/SECEX/CGEN   |

### RAFAEL DE SÁ MARQUES

O Secretário Executivo do Conselho de Gestão do Patrimônio Genético, em seu nome e no uso das competências que lhe foram conferidas pela Deliberação nº 203, de 19 de julho de 2007, com fundamento na Medida Provisória nº 2.186-16, de 23 de agosto de 2001, e no Decreto nº 3.945, de 28 de setembro de 2001, credencia como instituição fiel depositária de amostras de componente do patrimônio genético a Universidade Federal de Lavras - UFLA, nos seguintes termos:

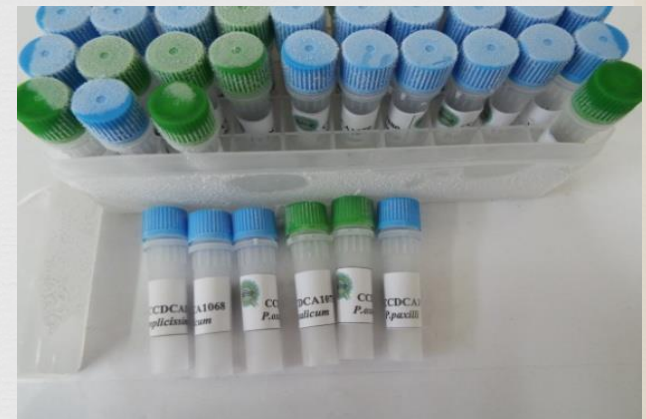
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| Nº do processo                    | 02000.002608/2014-16   |
| Instituição credenciada           | Universidade Federal de Lavras - UFLA                                |
| CNPJ                              | 22.078.679/0001-74   |
| Coleção                           | Coleção de Cultura do Departamento de Ciências dos Alimentos - CCDCA |
| Localidade da Coleção             | Lavras/MG  |
| Data da decisão de credenciamento | 5/11/2015  |
| Nº do credenciamento              | 141/2015/SECEX/CGEN  |

**Aviso de Credenciamento nº  
141/2015/SECEX/CGEN publicado no  
D.O.U. nº 219, de 17.11.2015, seção 3,  
página 129.**



*Aspergillus ochraceus*

**Coleção de Cultura de Microrganismos do  
Departamento de Ciência dos Alimentos  
Universidade Federal de Lavras  
CCDCA/UFLA**



**Tubos criogênicos a - 80°C**



# Microbioma: Microbiota terroir, em vinhos regionais



## ESTUDO EM DESENVOLVIMENTOS

### Metagenômica (Uva, Solo e Folhas)

Dissertação com previsão de defesa para 02/2018 de Lorena Dutra (Ciência dos Alimentos – UFFA)

COMMENTARY

## Microbial terroir for wine grapes

Jack A. Gilbert<sup>a,b,1</sup>, Daniel van der Lelie<sup>c</sup>, and Iratxe Zarraindia<sup>a,d</sup>

<sup>a</sup>Institute for Genomic and Systems Biology, Argonne National Laboratory, Argonne, IL 60439; <sup>b</sup>Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637; <sup>c</sup>Center for Excellence for Agricultural Biosolutions, FMC Corporation, Research Triangle Park, NC 27703; and <sup>d</sup>Ikerbasque, Basque Foundation for Science, 48011 Bilbao, Spain

The viticulture industry has been selectively growing vine cultivars with different traits (grape size, shape, color, flavor, yield of fruit, and so forth) for millennia, and small variations in soil composition, water management, climate, and the onset of vineyards have

and fungi, respectively, from grape must (freshly pressed grape juice, containing the skins and seeds) from plants in eight vineyards representing four of the major wine growing regions in California. The authors

presence of phytotoxic contaminants), to preventing the growth or activity of plant pathogens through competition for space and nutrients, antibiosis, production of hydrolytic enzymes, inhibition of pathogen-produced enzymes or toxins, and through systemic induction of plant defense mechanisms (3, 4). The most widely studied group of plant-associated microorganisms live in the soil surrounding the roots or inside the roots themselves (endophytes); the interface between roots and soil is often considered the key point of interaction between a plant and its

COMMENTARY

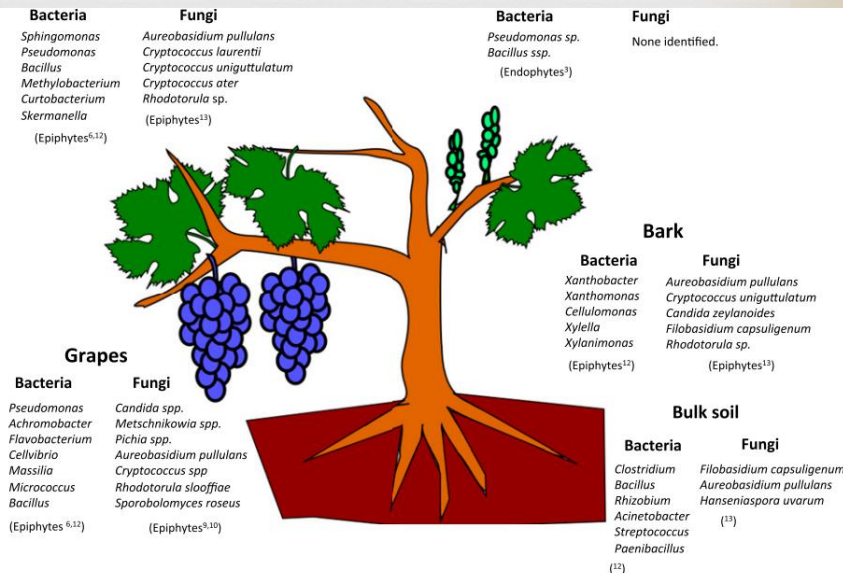


Fig. 1. Diagrammatic representation of some of characteristic bacteria and fungi known to show associations with the different tissues of *Vitis vinifera*.



# Associations among Wine Grape Microbiome, Metabolome, and Fermentation Behavior Suggest Microbial Contribution to Regional Wine Characteristics

Nicholas A. Bokulich,<sup>a,b,c\*</sup> Thomas S. Collins,<sup>b,d\*</sup> Chad Masarweh,<sup>a</sup> Greg Allen,<sup>e</sup> Hildegard Heymann,<sup>b</sup> Susan E. Ebeler,<sup>b,d</sup> David A. Mills<sup>a,b,c</sup>

Department of Food Science and Technology, University of California, Davis, California, USA<sup>a</sup>; Department of Viticulture and Enology, University of California, Davis, California, USA<sup>b</sup>; Foods for Health Institute, University of California, Davis, California, USA<sup>c</sup>; Food Safety and Measurement Facility, University of California, Davis, California, USA<sup>d</sup>; Far Niente and Nickel & Nickel Wineries, Oakville, California, USA<sup>e</sup>

\* Present address: Nicholas A. Bokulich, Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, Arizona, USA; Thomas S. Collins, Viticulture and Enology Program, Washington State University, Richland, Washington, USA.

**ABSTRACT** Regionally distinct wine characteristics (*terroir*) are an important aspect of wine production and consumer appreciation. Microbial activity is an integral part of wine production, and grape and wine microbiota present regionally defined patterns associated with vineyard and climatic conditions, but the degree to which these microbial patterns associate with the chemical composition of wine is unclear. Through a longitudinal survey of over 200 commercial wine fermentations, we demonstrate that both grape microbiota and wine metabolite profiles distinguish viticultural area designations and individual vineyards within Napa and Sonoma Counties, California. Associations among wine microbiota and fermentation characteristics suggest new links between microbiota, fermentation performance, and wine properties. The bacterial and fungal consortia of wine fermentations, composed from vineyard and winery sources, correlate with the chemical composition of the finished wines and predict metabolite abundances in finished wines using machine learning models. The use of postharvest microbiota as an early predictor of wine chemical composition is unprecedented and potentially poses a new paradigm for quality control of agricultural products. These findings add further evidence that microbial activity is associated with wine *terroir*.

**IMPORTANCE** Wine production is a multi-billion-dollar global industry for which microbial control and wine chemical composition are crucial aspects of quality. *Terroir* is an important feature of consumer appreciation and wine culture, but the many factors that contribute to *terroir* are nebulous. We show that grape and wine microbiota exhibit regional patterns that correlate with wine chemical composition, suggesting that the grape microbiome may influence *terroir*. In addition to enriching our un-

# Agradecimentos

