



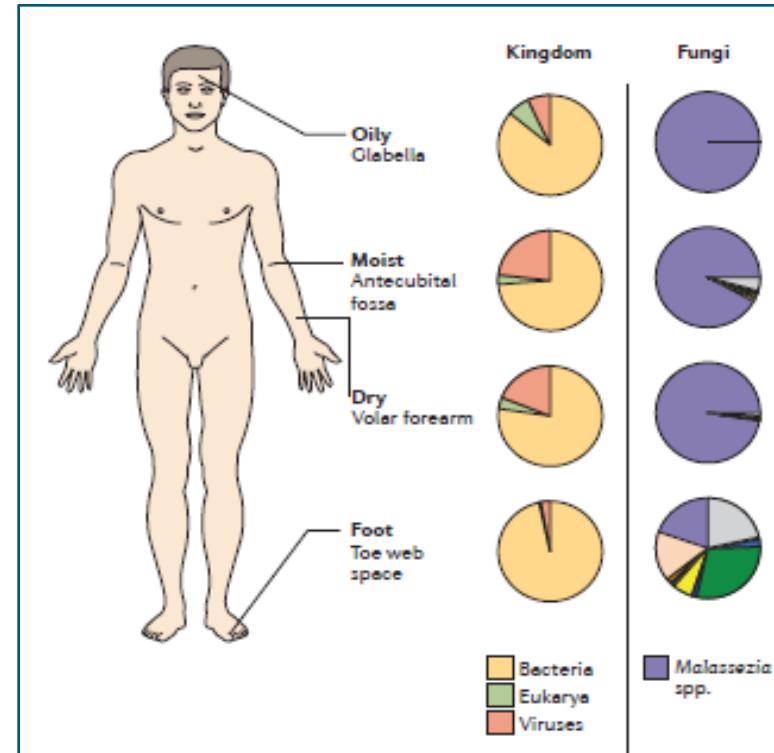
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**Horizontal gene transfer in
the skin fungus
Malassezia: NO resistance
is mediated by a
bacterially-derived
flavohemoglobin**

Giuseppe Ianiri, PhD
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The genus *Malassezia*

- Basidiomycetous yeasts
- 18 species
- Lipid-dependent
- Main fungus of the skin
- Commensal lifestyle
- Pathogenic lifestyle



Malassezia-associated clinical conditions

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Pityriasis versicolor



Atopic dermatitis



Dandruff

Cell Host & Microbe
Article

Cell Press

Malassezia Is Associated with Crohn's Disease and Exacerbates Colitis in Mouse Models

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<https://doi.org/10.1016/j.chom.2019.01.007>

LETTER

<https://doi.org/10.1038/s41586-019-1608-2>

The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL

Berk Aykur^{1,2}, Smriti Pushalkar^{2,3}, Ruinan Chen¹, Qianhao Li², Raquel Abenzerar⁴, Jacqueline I. Klim², Sorin A. Shadaloey⁵, Dongling Wu¹, Pamela Preisig¹, Narendra Verma², Yuqi Guo², Anjana Saxena^{4,6}, Mridula Vardhan², Brian Diskin¹, Wei Wang², Joshua Leifwand¹, Emma Kunz¹, Juan A. Kochen Rossi¹, Mawtin Hundley¹, Constantinos Zambrini¹, Xin Li², Deepak Saxena^{1,2,*} & George Miller^{1,2,*}

Malassezia genome research

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- Small genomes (~7-9 Mb)
- Absence of fatty acid synthase
- Loss of RNAi
- Loss of enzymes involved in carbohydrate metabolism and hydrolysis
- 44 *Malassezia*-specific gene clusters



RESEARCH ARTICLE

Genus-Wide Comparative Genomics of *Malassezia* Delineates Its Phylogeny, Physiology, and Niche Adaptation on Human Skin

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- Jun Xu et al. 2007. PNAS vol. 104 no. 47, 18730–18735. doi: 10.1073/pnas.0706756104
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Agrobacterium tumefaciens-mediated transformation of *Malassezia*

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Gene Function Analysis in the Ubiquitous Human Commensal and Pathogen *Malassezia* Genus

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RESEARCH ARTICLE

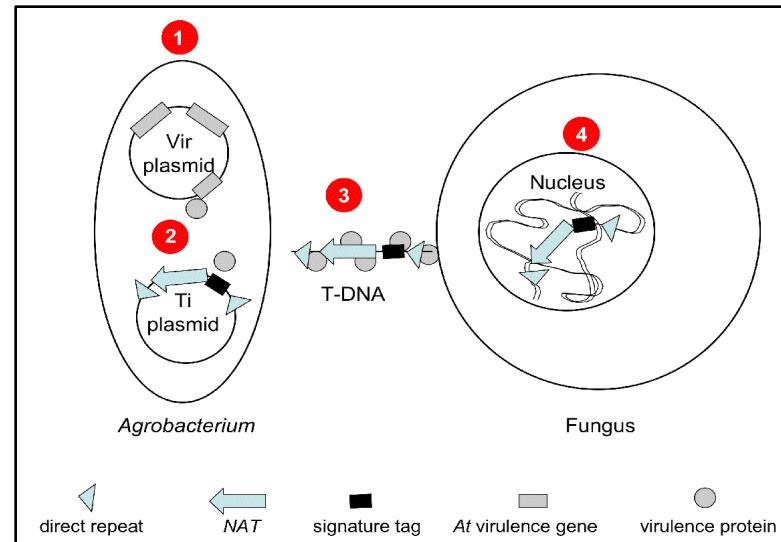


HIGHLIGHTED ARTICLE
GENETICS | INVESTIGATION

Advancing Functional Genetics Through *Agrobacterium*-Mediated Insertional Mutagenesis and CRISPR/Cas9 in the Commensal and Pathogenic Yeast *Malassezia*

Giuseppe Ianiri, Gabriel Dagotto, Sheng Sun, and Joseph Heitman¹

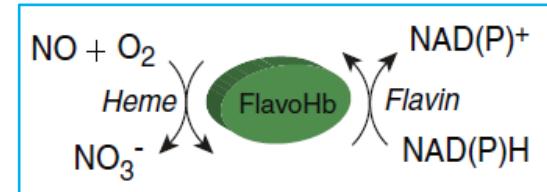
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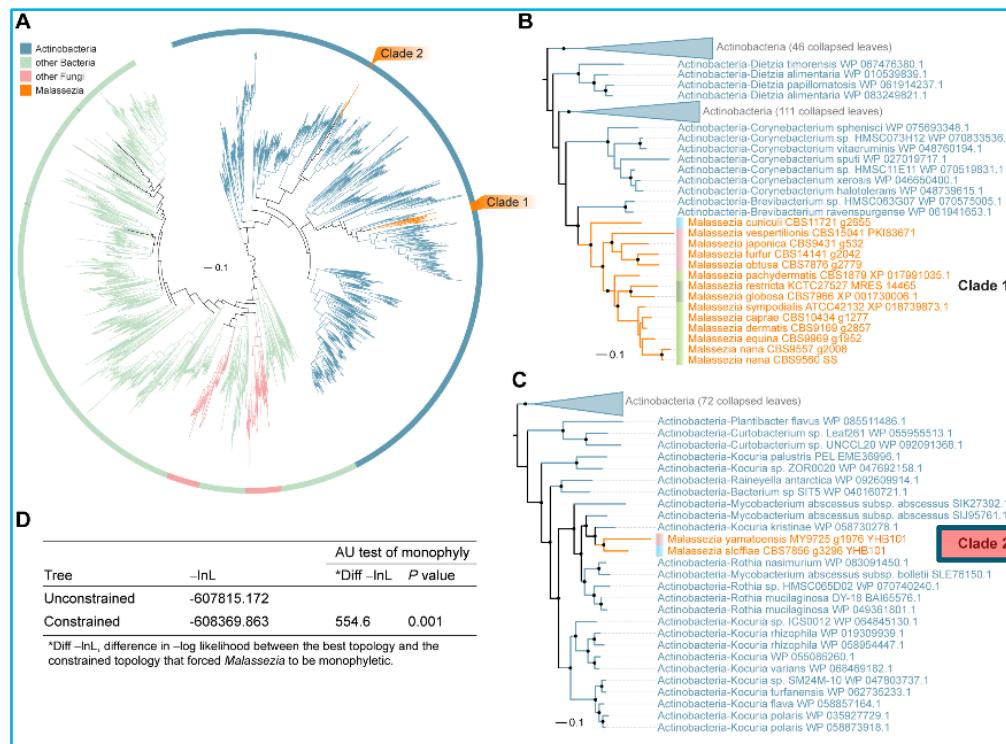
Role of flavohemoglobin in bacteria, fungi and protozoa

Organism	Gene name	Model	Consequence of knockout (i.e., function)	Refs.
Bacteria				
<i>Escherichia coli</i> (nonpathogenic)	<i>hmpA</i>	Exogenous NO, nitrate respiration	NO hypersensitivity (decreased growth, respiratory inhibition, increased SNOs)	[18,42,43,79,149]
<i>E. coli</i> (uropathogenic)	<i>hmpA</i>	Exogenous NO, elevated FlavoHb in virulent human isolates	NO hypersensitivity	[126,150]
<i>Salmonella typhimurium</i>	<i>hmpA</i>	Exogenous NO, anaerobic respiration, macrophage phagocytosis, murine intraperitoneal infection	NO hypersensitivity, decreased virulence, elevated SNOs, hyperfilamentation, amino acid auxotrophy	[19,80,94,151]
<i>Pseudomonas aeruginosa</i>	<i>hmp</i>	Exogenous NO	NO hypersensitivity	[66]
<i>Staphylococcus aureus</i>	<i>hmp</i>	Exogenous NO (microaerophilic)	NO hypersensitivity	[152]
<i>Bacillus subtilis</i>	<i>hmp</i>	Exogenous NO, nitrate respiration	NO hypersensitivity, poor long-term survival	[67,153,154]
<i>Yersinia pestis</i>	<i>hmp</i>	Exogenous NO, intradermal infection, bubo formation	NO hypersensitivity, decreased virulence	[155]
<i>Vibrio fischeri</i>	<i>hmp</i>	Exogenous NO, squid colonization	NO hypersensitivity, decreased squid colonization, decreased cellular aggregation	[85]
<i>Vibrio cholerae</i>	<i>hmp</i>	Exogenous NO, murine gut colonization	NO hypersensitivity, decreased gut colonization, decreased pathogenicity	[156]
<i>Sinorhizobium meliloti</i> <i>(Rhizobium)</i>	<i>hmp</i>	Exogenous NO, symbiotic denitrification	NO hypersensitivity, attenuated denitrification, regulation of symbiosis and nodulation	[157,158]
<i>Erwinia chrysanthemi</i>	<i>hmpX</i>	Exogenous NO, plant infection assays	NO hypersensitivity, impaired pathogenicity in model of plant infection	[159,160]
<i>Alcaligenes eutrophus</i>	<i>hmp</i>	Denitrification	Prevents N ₂ O release	[161]
<i>Streptomyces antibioticus</i>	None	Exogenous NO	Unknown	[73]
Eukarya				
<i>Saccharomyces cerevisiae</i>	<i>yhb1</i>	Exogenous NO	NO hypersensitivity, decreased mitochondrial respiration, hyperfilamentous, elevated mitochondrial NO synthesis	[72,81,95,97,162]
<i>Candida albicans</i>	<i>yhb1, yhb4, yhb5</i>	Exogenous NO, murine fungemia/infection	NO hypersensitivity, decreased virulence	[163]
<i>Cryptococcus neoformans</i>	<i>ftb1</i>	Exogenous NO, macrophage killing	NO hypersensitivity, decreased virulence	[129,131]
<i>Aspergillus oryzae</i>	<i>ftbA, ftbB</i>	Exogenous NO	NO hypersensitivity, regulation of hyphal growth	[82,164,165]
<i>Aspergillus nidulans</i>	<i>ftbA, ftbB</i>	Exogenous NO, NO ₃ ⁻ assimilation	NO hypersensitivity, impaired nitrate assimilation, enhanced NO-mediated sexual development	[166-168]
<i>Botrytis cinerea</i>	<i>ftg1</i>	Exogenous NO	NO hypersensitivity, maintenance of conidia	[169]
<i>Dictyostelium discoideum</i>	<i>fta, ftb</i>	Exogenous NO	NO hypersensitivity, cellular size regulation	[170]
<i>Giardia intestinalis</i>	<i>hmpA</i>	Exogenous NO	Unknown	[171]
<i>Giardia lamblia</i>	<i>hmpA</i>	Exogenous NO	Unknown	[172]

The table is organized by each particular organism, along with experimental model and function (if known). Abbreviations: NO, nitric oxide; SNO, S-nitrosothiol.



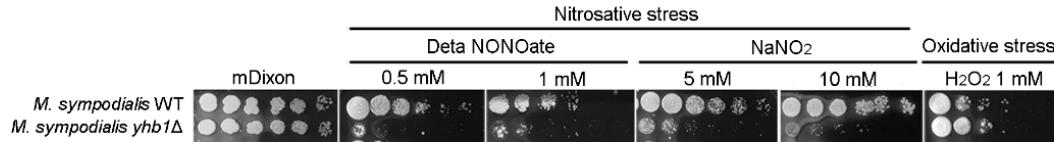
Flavohemoglobin-encoding genes were acquired by *Malassezia* through independent HGT events from *Actinobacteria*



- Hoogewijs D, et al., 2012. PLoS ONE 7(2): e31856
- Visecaver et al., 2016. Mol Biol Evol. Aug;33(8):1979-87

Malassezia flavohemoglobins are involved in resistance to nitrosative stress and NO degradation

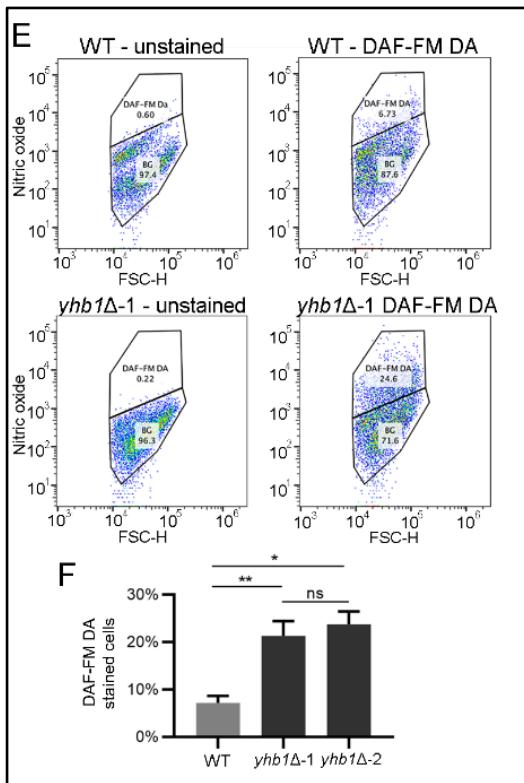
A



CLADE 2 flavohemoglobin Yhb101
from *M. yamatoensis*

Transcriptomic profile of *M. sympodialis* in presence of NO accumulation

DAF-FM DA = fluorescent signal in the presence of NO



Flavohemoglobin is involved in macrophage survival and *in vivo* virulence in *Cryptococcus neoformans* and *Candida albicans*, but not in *Aspergillus fumigatus*

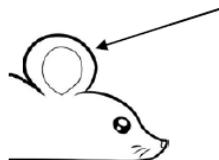
Cell Host & Microbe
Article CellPress

The Skin Commensal Yeast *Malassezia* Triggers a Type 17 Response that Coordinates Anti-fungal Immunity and Exacerbates Skin Inflammation

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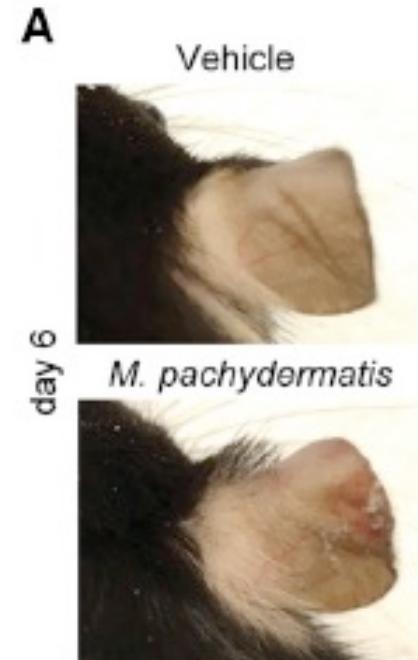
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<https://doi.org/10.1016/j.chom.2019.02.002>

The infection model:



1. mild tape-stripping
2. epicutaneous application of
 - vehicle (olive oil)
 - 2 OD₆₀₀ *Malassezia* spp. (in olive oil)

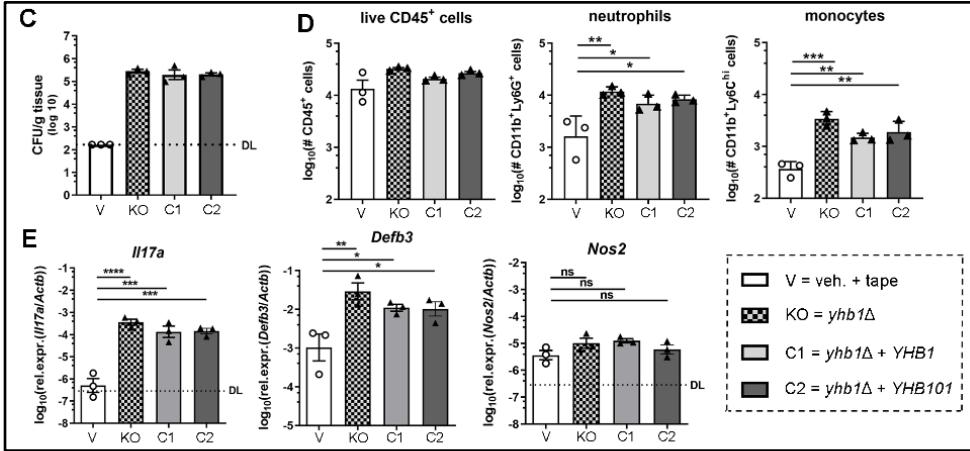
WT C57BL/6 mice



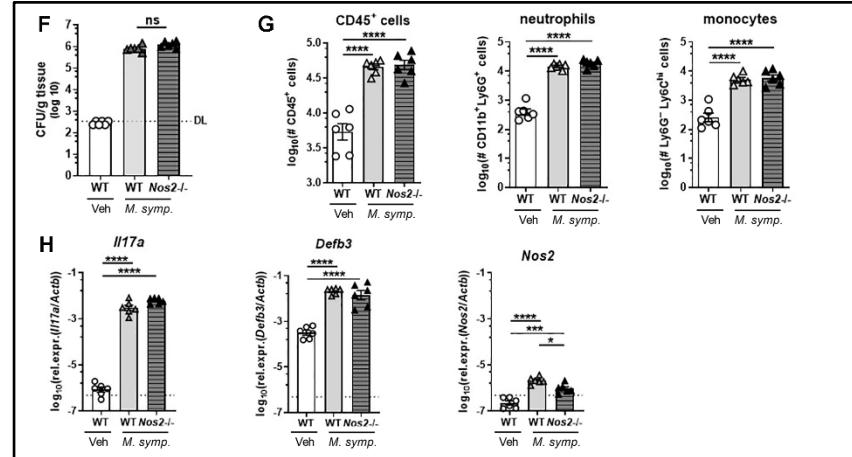
Malassezia flavohemoglobins are not required for pathogenesis in ex vivo and in vivo models.

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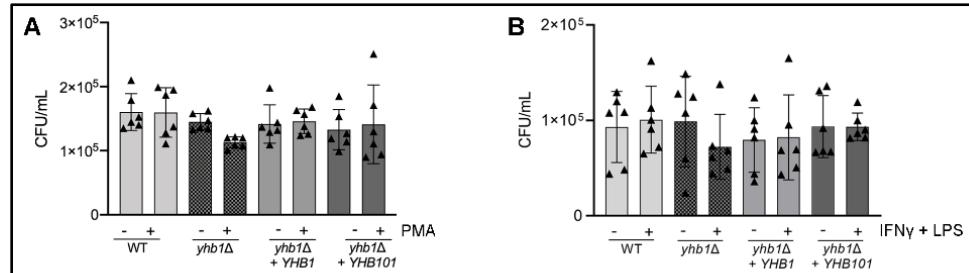
In vivo - WT C57BL/6j mice



In vivo - *iNos2*-/- mice



Ex vivo - J774A.1 cells



Additional HGT events in *Malassezia*



- Visecaver et al., 2016. Mol Biol Evol. 33(8):1979-87
- Alexander et al. 2016 PNAS 113, 4116-4121

- *Malassezia* acquired flavohemoglobin-encoding genes through independent HGT events from Actinobacteria, which are part of the natural microbiome and thus are sympatric with *Malassezia* fungi
- Both flavohemoglobins actively consume NO to counteract nitrosative stress
- Accumulation of intracellular NO leads to upregulation of stress response genes and downregulation of predicted pathogenicity factors
- *In vivo* pathogenesis is independent of *Malassezia* flavohemoglobin
- Several other species-specific HGT events have occurred and might have contributed to *Malassezia* evolution and speciation



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Laboratory

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New Results

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Horizontal gene transfer in the human and skin commensal *Malassezia*: a bacterially-derived flavohemoglobin is required for NO resistance and host interaction

Giuseppe Ianiri, Marco A. Coelho, Fiorella Ruchti, Florian Sparber, Timothy J. McMahon, Ci Fu, Madison Bolejack, Olivia Donovan, Hayden Smutney, Peter Myler, Fred Dietrich, David Fox III, Salomé LeibundGut-Landmann, Joseph Heitman

doi: <https://doi.org/10.1101/2020.01.28.923367>

This article is a preprint and has not been certified by peer review [what does this mean?].

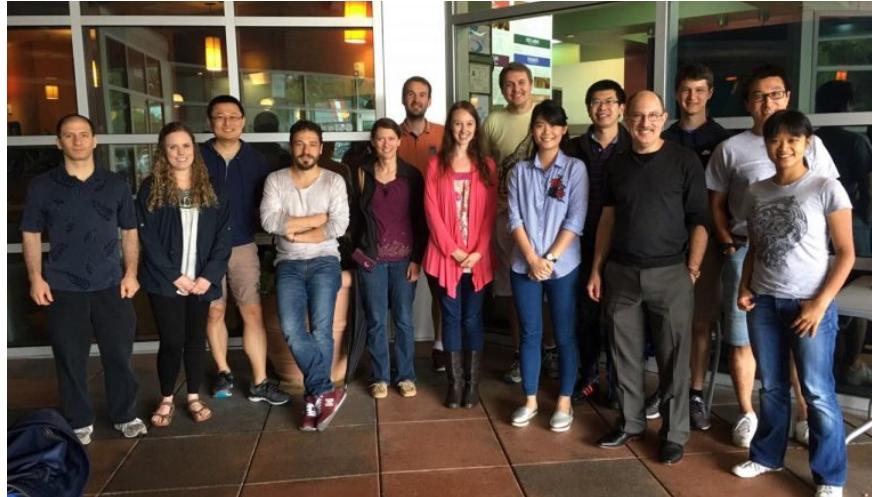
Acknowledgements

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- Dr. Marco Coelho (Bioinformatics, Heitman Lab)
- Dr. Florian Sparber, Fiorella Ruchti and Prof. Salomè LeibundGut-Landmann (murine skin model, University of Zurich)
- Dr. David Fox III (Crystallography, UCB Seattle)
- Prof. Fred Dietrich (Bioinformatics, Duke MGM)
- Prof. Tim McMahon (NO metabolism, Duke Medicine)



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National Institute of
Allergy and
Infectious Diseases

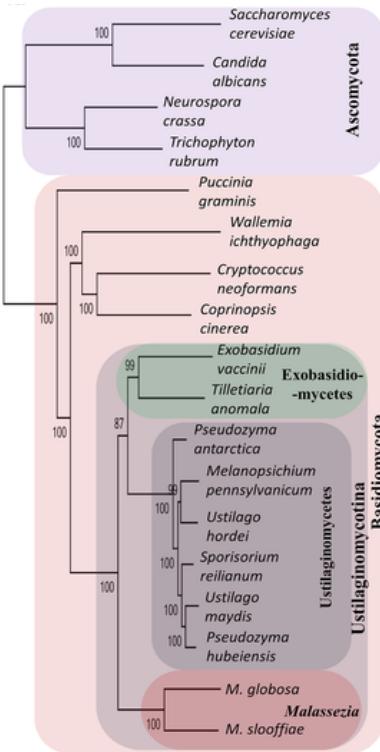


QUESTIONS?

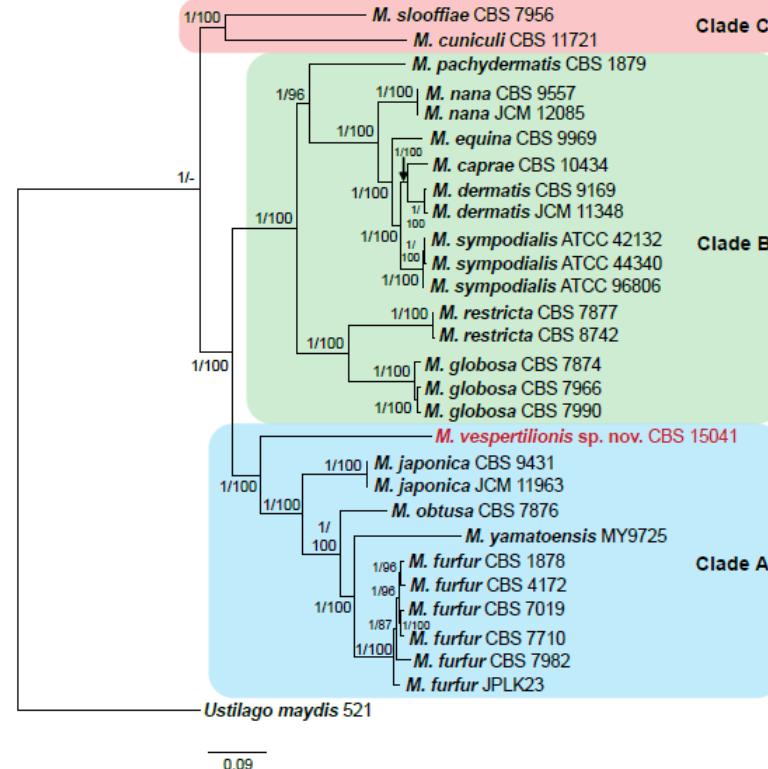
Taxonomy of *Malassezia*

- More phylogenetically related to the Basidiomycota plant pathogens *Ustilago maydis* and *U. hordei*
- Highly divergent from other Ascomycota fungi that live on the skin (i.e. *Candida* species and dermatophytes)

Fungal phylogeny



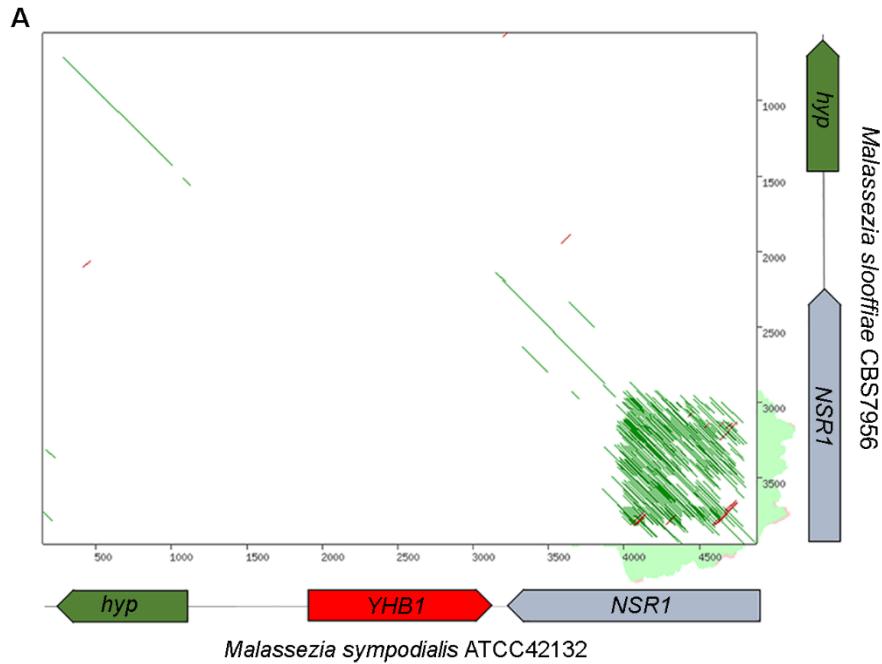
Malassezia species phylogeny



Outliers

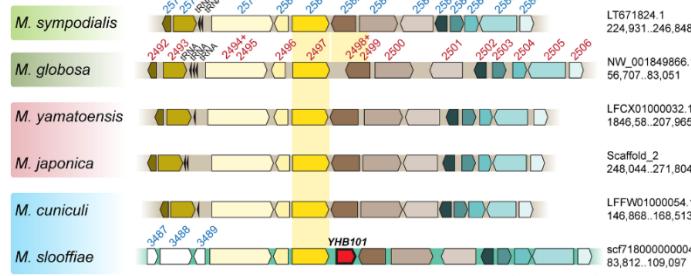
Commensal species

Fungemia-causing species

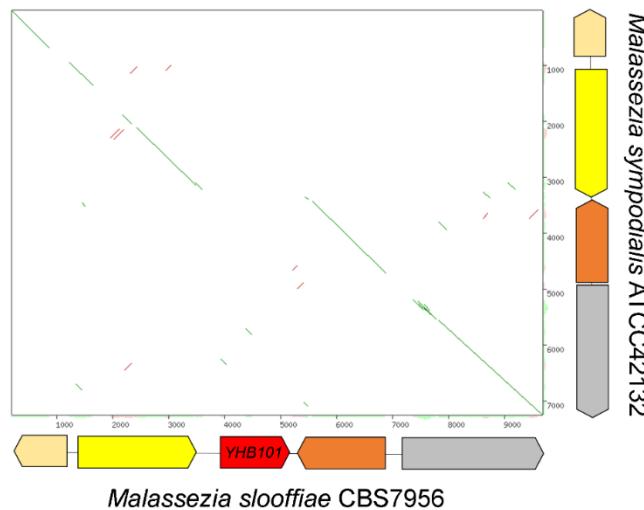


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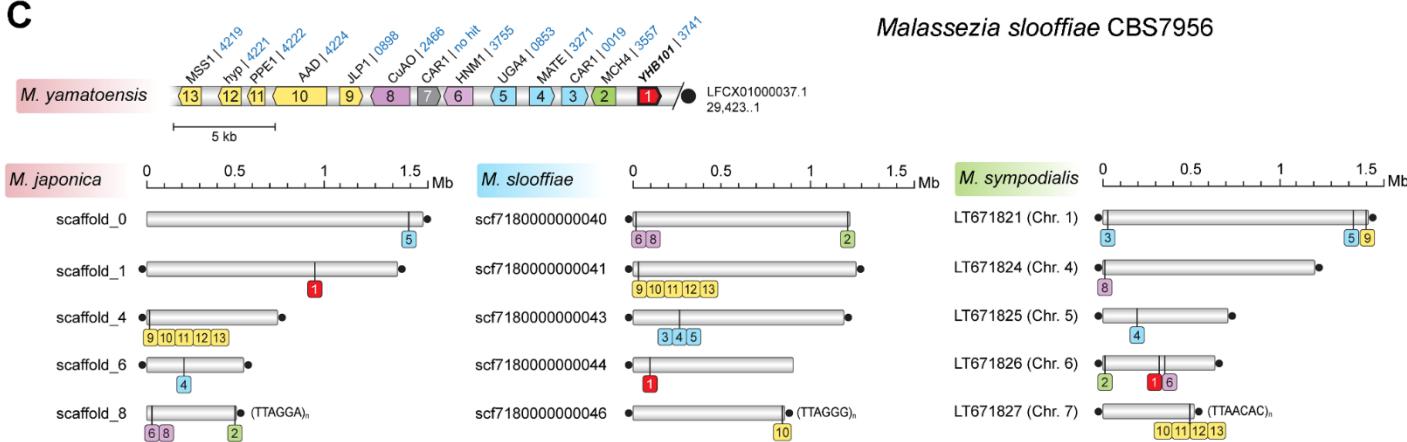
A



B



C



Development of a NO consume assay for *Malassezia*

Biological activity of nitric oxide in the plasmatic compartment

Xunde Wang*, Jose E. Tanus-Santos*, Christopher D. Reiter*, Andre Dejam*, Sruti Shiva*, Reginald D. Smith*, Neil Hogg*, and Mark T. Gladwin**†||

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