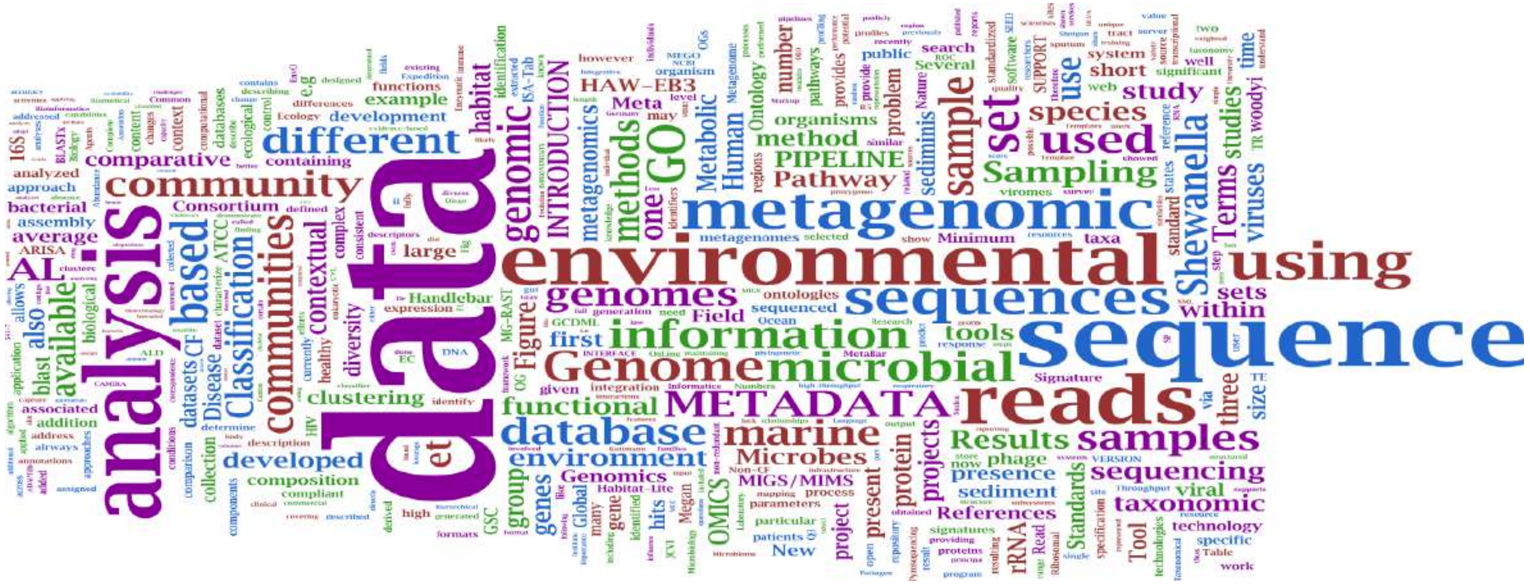
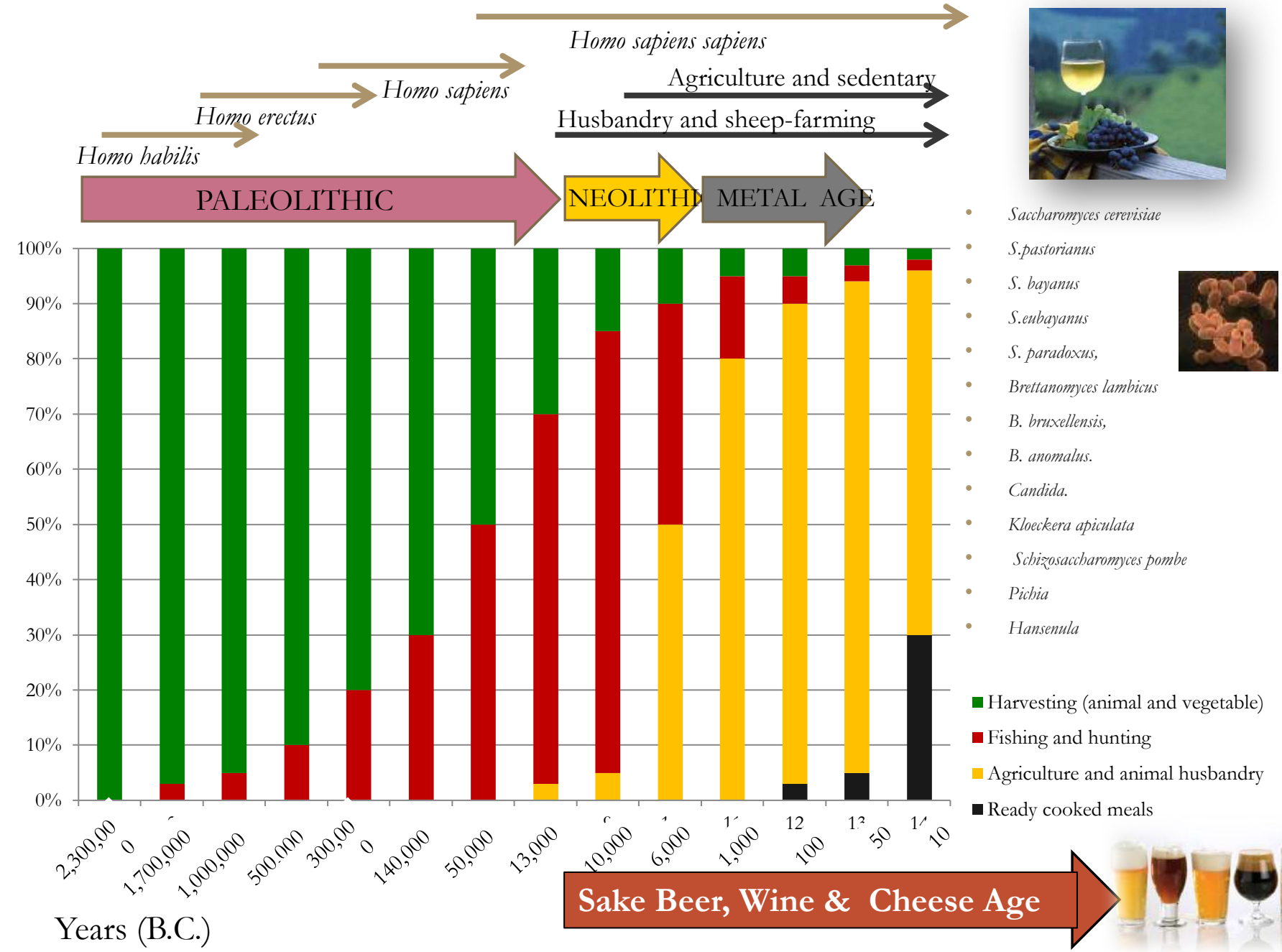
Il Micobiota: I lieviti in noi

Prof. Duccio Cavalieri

www.duccioknights.org,
Cavalieri.unifi@gmail.com



Evolution of human diet

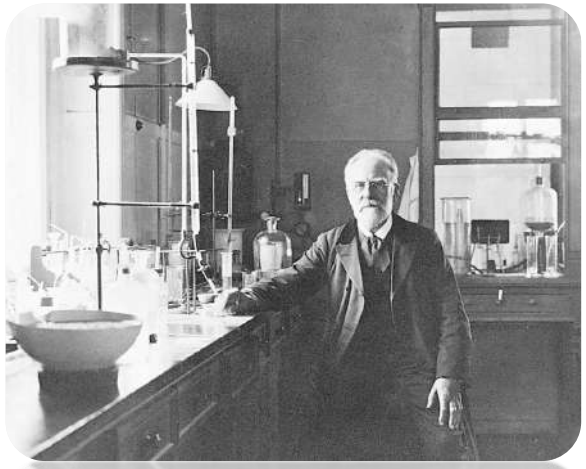


- *Saccharomyces cerevisiae*
- *S.pastorianus*
- *S. bayanus*
- *S.eubayanus*
- *S. paradoxus,*
- *Brettanomyces lambicus*
- *B. bruxellensis,*
- *B. anomalus.*
- *Candida.*
- *Kloeckera apiculata*
- *Schizosaccharomyces pombe*
- *Pichia*
- *Hansenula*

On the shoulder of giants….

- 1876 – “etudes sur la levure de la biere” Louis Pasteur
  - Yeast is the actor of alcoholic fermentation

- 1874 –Jacobsen brings Emil Christian Hansen under the hill of Carl (Carlsberg Labs)
  - Pure culture of S.pastorianus
  - 1883 – Buchner-Co2 and Etoh produced by yeast



Louis Pasteur 1871-"Il n‘existe pas une catégorie de sciences auxquelles on puisse donner le nom de sciences appliquées. Il y a la science et les applications de la science, liées entre elles comme le fruit à l‘arbre qui l‘a porté”

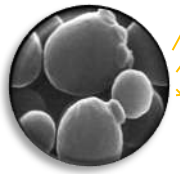
Yeast- WASP Lab

Irene Stefanini

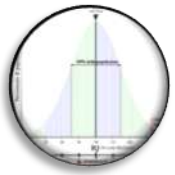
Lisa Rizzetto

Niccolò Meriggi

Francesco Vitali



Carlotta de Filippo

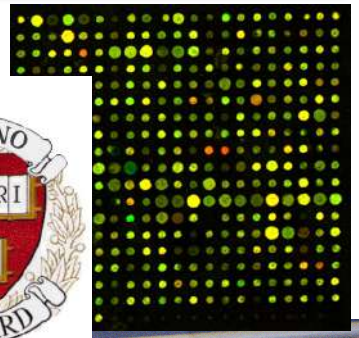


Leonardo Dapporto

Stefano Turillazzi

Valentina Cappelletti

Mario Polsinelli



Keith Morneau,, J. S.Oh, J. W. Zhao, Juk Fai Leung, Agnes Bergerat

P. Grosu

Bauer Center for Genomics Research, Harvard university

Role of social wasps in *Saccharomyces cerevisiae* ecology and evolution

Irene Stefanini[a,1], Leonardo Dapporto[b,c,1], Jean-Luc Legras[d,e,f], Antonio Calabretta[a,b], Monica Di Paola[g], Carlotta De Filippo[h], Roberto Viola[h], Paolo Capretti[c], Mario Polsinelli[b], Stefano Turillazzi[b,i], and Duccio Cavalieri[a,h,2]

Nest foundation

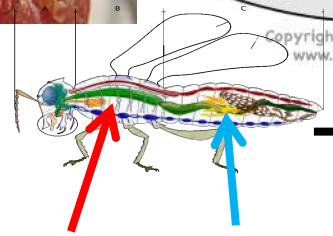
hibernation

mating

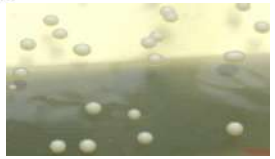
sexuals

workers

Vespa crabro
Polistes sp.



Crop and Gut dissection

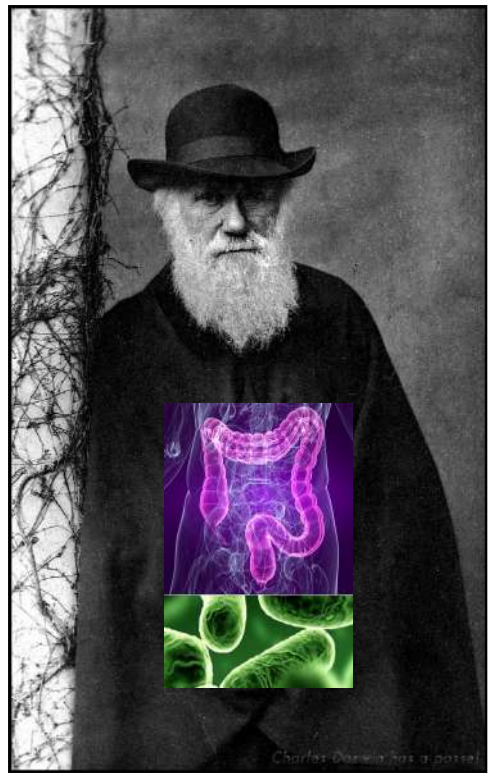


Yeasts isolation and characterization

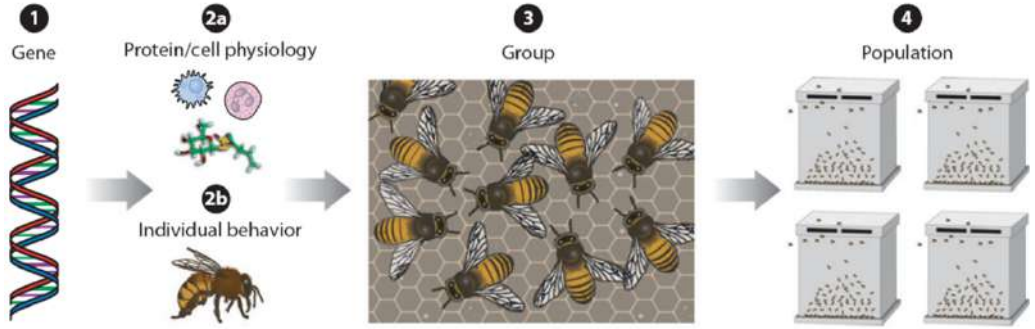
S. cerevisiae identification

What is the advantage for the host to harbor *S. cerevisiae*?

- **Defence against pathogens and production of antimicrobial substances (wasps, ants)**



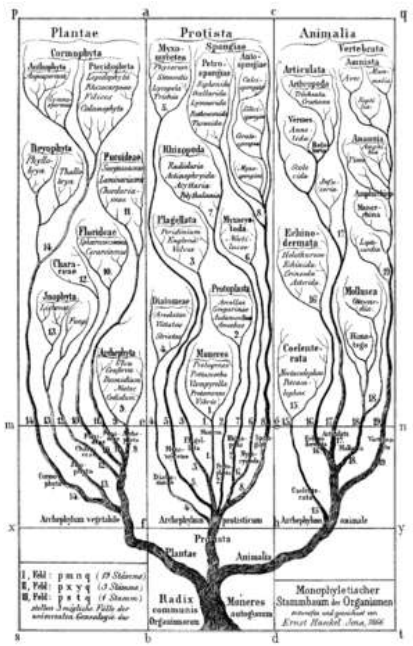
- **Social immunity**
  - **Honey bees**
  - **ants**



The host and his microbiome are the unit of selection

(Zilber-Rosenberg I. & Rosenberg E)

Metagenomics 2005-now



Untargete Metagenomics
DNA-RNA SEQ

Targeted Metagenomics
Sequencing Clock genes

De Novo Assembly

Novel functions eco-meta biology

Phyla

Genera

Species

Annotated Genes

Public DBs

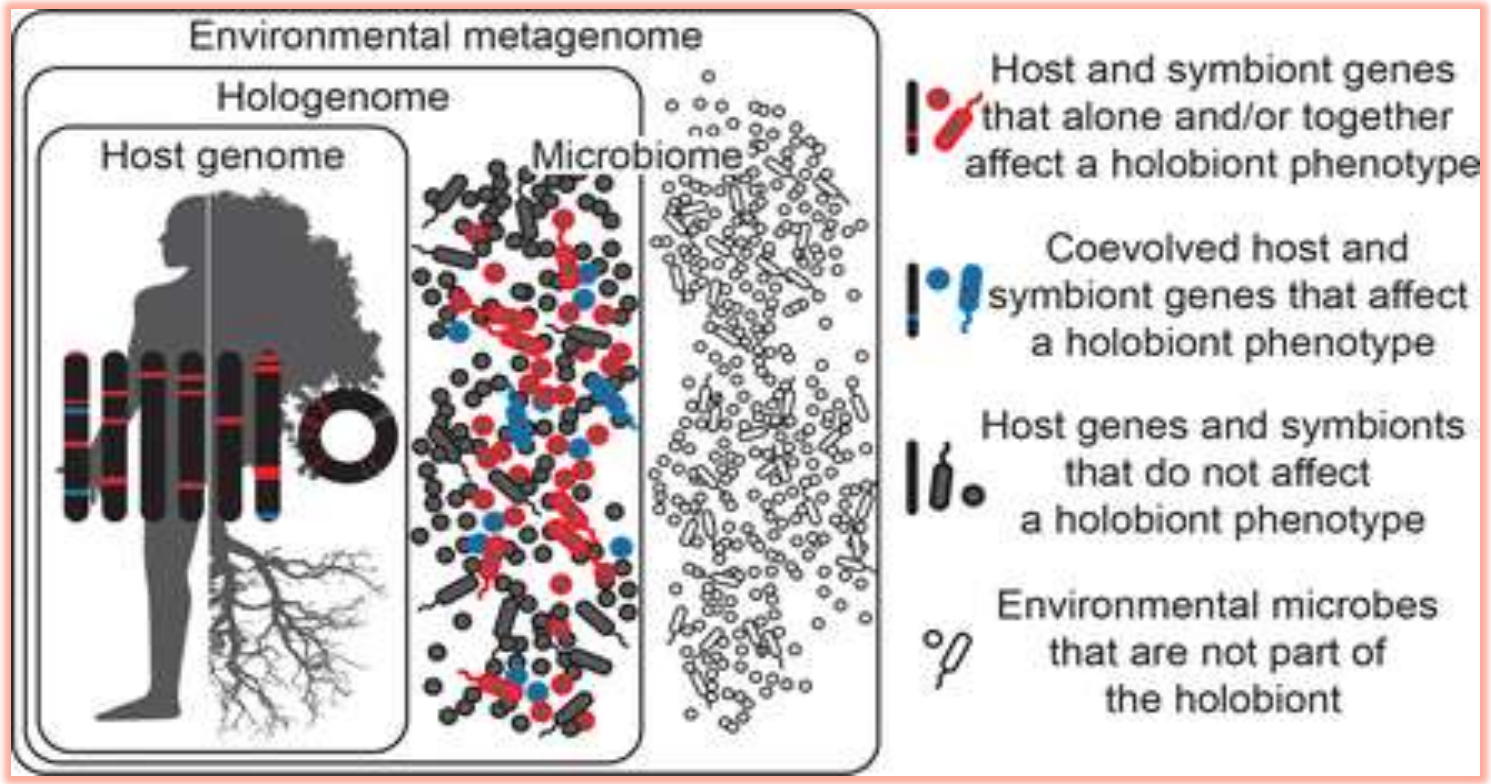
LIMS-Meta analysis
GBC-ISA

Population structure and ecology of microbial fluxes

The hologenome

Hologenome encompasses the genomes of the host and all of its microorganisms (holobiont).

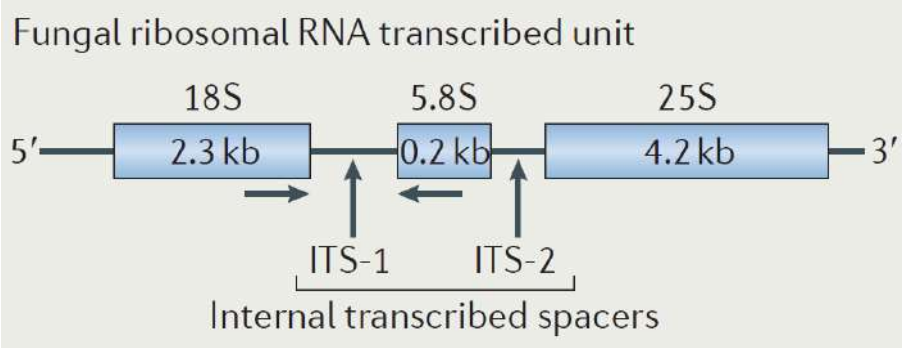
Microorganisms in the environment are not part of the holobiont but can affect the complexity and stability of hologenome.

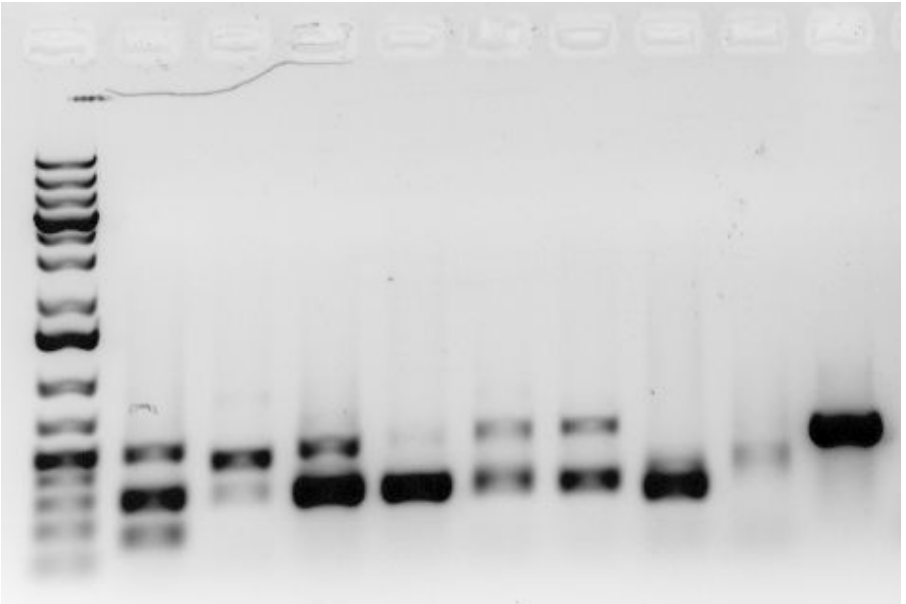


Theis KR et al. *mSystems* 2016

Solo Batteri? E i Lieviti?

Metagenomics of the human gut mycobiota





ITS regions of the fungal rDNA targeted for mycobiome characterization

- In fungi, the locus is typically duplicated 100–200 times
- highly divergent between fungi (*in sequence and length*)
- Sexual dimorphism in fungal taxonomy
- It is present in all eukaryotes (possibility of contaminant seqs)

Isolation, Identification and Characterization of Yeasts from Fermented Goat Milk of the Yaghnob Valley in Tajikistan

*Linnea A. Qvirist[1], Carlotta De Filippo[2], Francesco Strati[3], Irene Stefanini[3], Maddalena Sordo[3], Thomas Andlid[1], Giovanna E. Felis[4], Paola Mattarelli[5] and Duccio Cavalieri[6]**

[1] Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden, [2] Institute of Biometeorology, National Research Council, Florence, Italy, [3] Department of Computational Biology, Edmund Mach Foundation, San Michele all'Adige, Italy, [4] Department of Biotechnology, University of Verona, Verona, Italy, [5] Department of Agricultural Sciences, University of Bologna, Bologna, Italy, [6] Department of Biology, University of Florence, Florence, Italy

The geographically isolated region of the Yaghnob Valley, Tajikistan, has allowed its inhabitants to maintain a unique culture and lifestyle. Their fermented goat milk constitutes one of the staple foods for the Yaghnob population, and is produced by backslopping, i.e., using the previous fermentation batch to inoculate the new one. This study addresses the yeast composition of the fermented milk, assessing genotypic, and phenotypic properties. The 52 isolates included in this study revealed small species diversity, belonging to *Kluyveromyces marxianus*, *Pichia fermentans*, *Saccharomyces cerevisiae*, and one *Kazachstania unispora*. The *K. marxianus* strains showed two different genotypes, one of which never described previously. The two genetically different groups also differed significantly in several phenotypic characteristics, such as tolerance toward high temperatures, low pH, and presence of acid. Microsatellite analysis of the *S. cerevisiae* strains from this study, compared to 350 previously described strains, attributed the Yaghnobi *S. cerevisiae* to two different ancestry origins, both distinct from the wine and beer strains, and similar to strains isolated from human and insects feces, suggesting a peculiar origin of these strains, and the existence of a gut reservoir for *S. cerevisiae*. Our work constitutes a foundation for strain selection for future applications as starter cultures in food fermentations. This work is the first ever on yeast diversity from fermented milk of the previously unexplored area of the Yaghnob Valley.

Keywords: yeast, fermented goat milk, Yaghnob Valley Tajikistan, identification, phenotyping, genotyping

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Specialty section:
This article was submitted to Food Microbiology, a section of the journal Frontiers in Microbiology

Candida albicans Is Not Always the Preferential Yeast Colonizing Humans: A Study in Wayampi Amerindians

Cécile Angebault,[1,2,3,6,7] Félix Djossou,[8] Sophie Abélanet,[3,6] Emmanuelle Permal,[3,6] Mouna Ben Soltana,[2] Laure Diancourt,[5] Christiane Bouchier,[4] Paul-Louis Woerther,[1] François Catzeflis,[9] Antoine Andremont,[1] Christophe d'Enfert,[3,6] and Marie-Elisabeth Bougnoux[2,3,6,7]

Population genomics of *Saccharomyces cerevisiae* human isolates: passengers, colonizers, invaders

Running title: Host-yeast co-evolution in the human gut

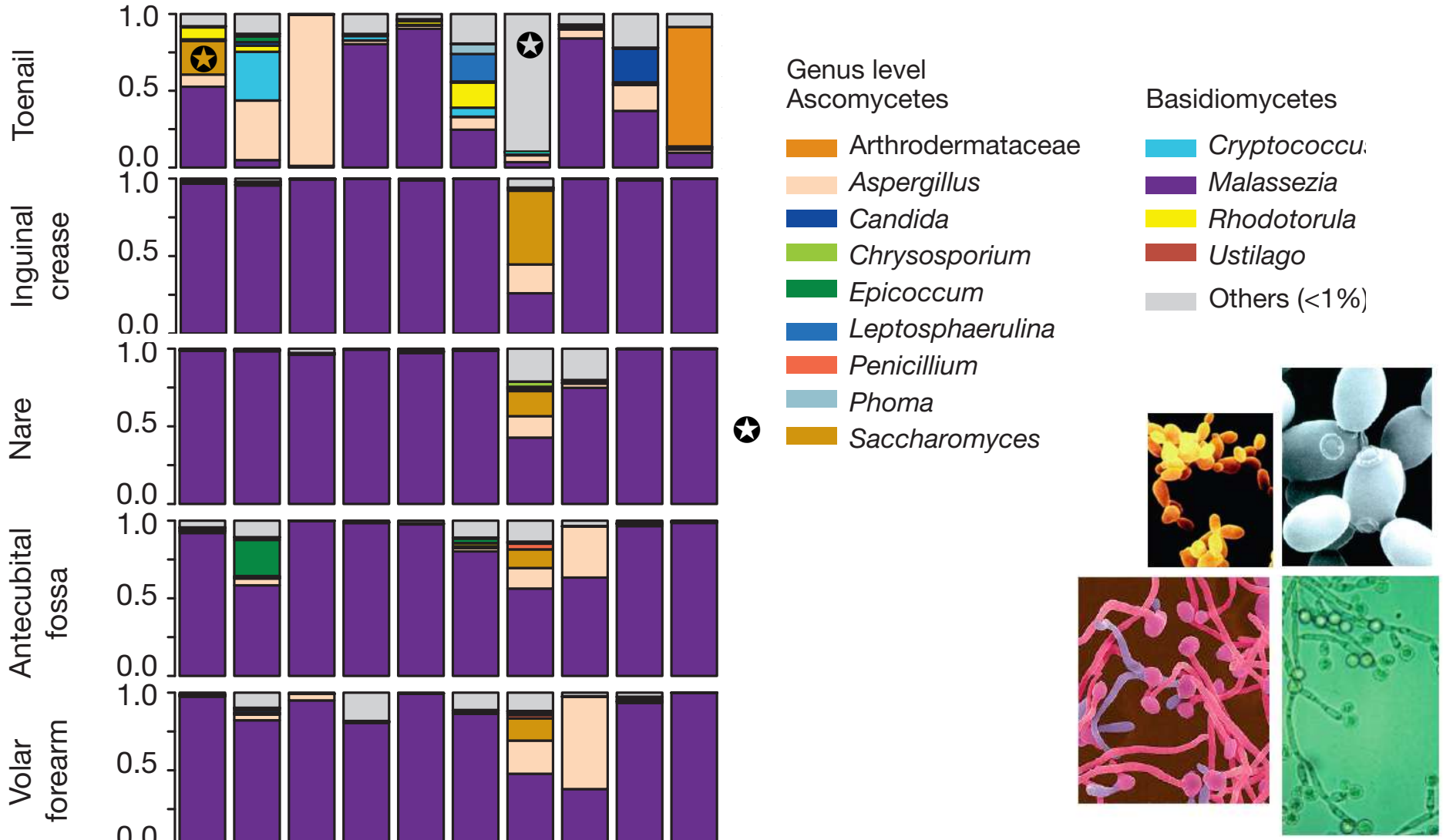
Carlotta De Filippo[1,10], Monica Di Paola[2,10], Irene Stefanini[1], Lisa

LETTER

doi:10.1038/nature12171

Topographic diversity of fungal and bacterial communities in human skin

Keisha Findley[1], Julia Oh[1], Joy Yang[1], Sean Conlan[1], Clayton Deming[1], Jennifer A. Meyer[1], Deborah Schoenfeld[2], Effie Nomicos[2], Morgan Park[3], NIH Intramural Sequencing Center Comparative Sequencing Program†, Heidi H. Kong[2]* & Julia A. Segre[1]*



Role of the Mycobiome in Human Acute Graft-versus-Host Disease

Walter J.F.M. van der Velden [1,3,*], Mihai G. Netea [2,3], Anton F.J. de Haan [4], Gerwin A. Huls [1], J. Peter Donnelly [1,3], Nicole M.A. Blijlevens [1,3]

Obesity changes the human gut mycobiome

M. Mar Rodríguez[1], Daniel Pérez[2], Felipe Javier Chaves[2], Eduardo Esteve[1], Pablo Marin-Garcia[2], Gemma Xifra[1], Joan Vendrell[3], Mariona Jové[4], Reinald Pamplona[4], Wifredo Ricart[1], Manuel Portero-Otin[4], Matilde R. Chacón[3,*] & José Manuel Fernández Real[1,*]

Dysbiosis of Fungal Microbiota in the Intestinal Mucosa of Patients with Colorectal Adenomas

Chunguang Luan[1,2], Lingling Xie[3], Xi Yang[1,2], Huifang Miao[4], Na Lv[1], Ruifen Zhang[1], Xue Xiao[1], Yongfei Hu[1], Yulan Liu[3], Na Wu[3], Yuanmin Zhu[3] & Baoli Zhu[1,5]

International Journal of Clinical Medicine, 2013, 4, 309-312
doi:10.4236/ijcm.2013.47054 Published Online July 2013 (http://www.scirp.org/journal/ijcm)

Scientific Research

A Case Study of Gut Fermentation Syndrome (Auto-Brewery) with *Saccharomyces cerevisiae* as the Causative Organism

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Received April 25th, 2013; revised May 30th, 2013; accepted June 12th, 2013

ABSTRACT

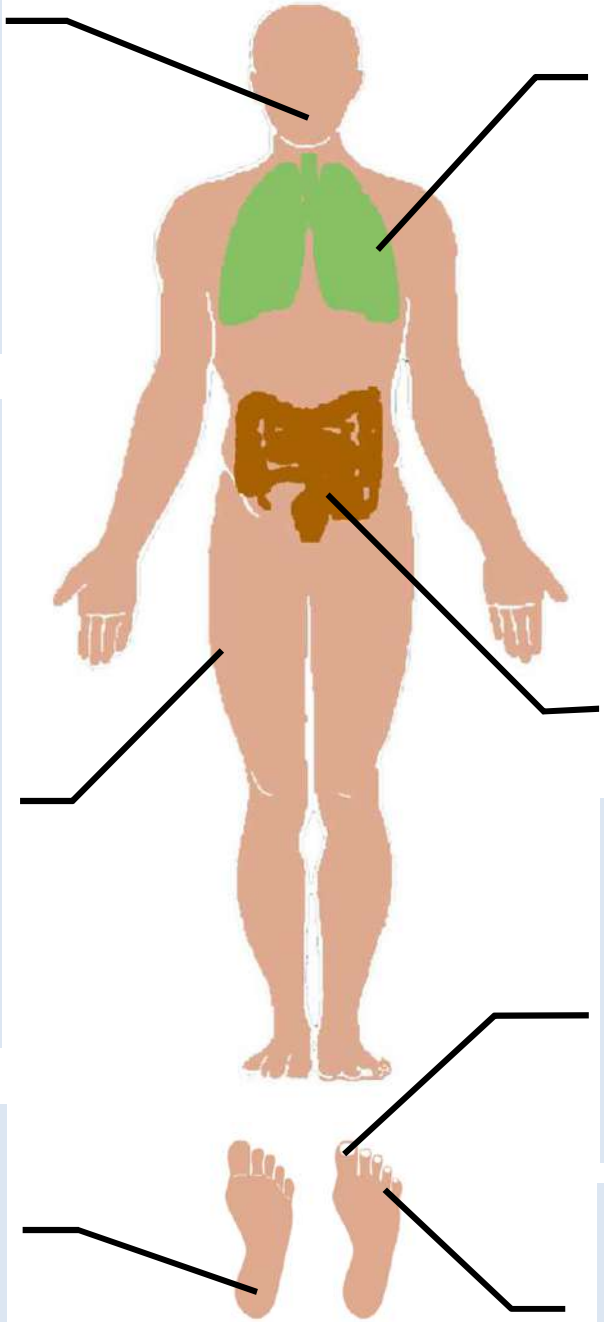
Gut Fermentation Syndrome also known as Auto-Brewery Syndrome is a relatively unknown phenomenon in modern medicine. Very few articles have been written on the syndrome and most of them are anecdotal. This article presents a case study of a 61 years old male with a well documented case of Gut Fermentation Syndrome verified with glucose and carbohydrate challenges. Stool cultures demonstrated the causative organism as *Saccharomyces cerevisiae*. The patient was treated with antifungals and a low carbohydrate diet and the syndrome resolved. *Helicobacter pylori* was also found and could have been a possible confounding variable although the symptoms resolved post-treatment of the *S. cerevisiae*.

The human mycobiota

abundant genus in the oral mucosa the **oral mucosa.**
Other fungi present are ***Penicillum***, *Aspergillus spp., Scopulariopsis, Homodendrum, Cladosporium, Saccharomycetales, Fusarium, Cryptococcus*

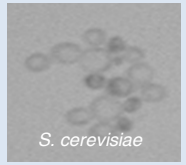
Several ***Malassezia*** species *(M. globosa, M. restricta, M. sympodialis*) are the most abundant in the **skin** (and also in **nare**).
Other species present are: ***Rhodoturola***, *Candida* (*C. albicans, C. diffluens, C. liquefacens*), *Aspergillus* (*A. candidus, A. terreus, A. versicolor*), *Epicoccum* and at lower abundance *Alternaria, Penicillium , Cladosporium, Mucor, Tricophyto*n.

Malassezia and ***Aspergillus*** genera are commonly inhabitant of **plantar heel**.
Others are *Epiccocum, Leptosphaerulina, Phoma, Candida*, and *Cryptococcus*. In lower abundance, *Rhodoturola* and



domitate the **lung** mycobiota.
In CF patients, *C. albicans, C. paraspilosis* and *Criptococcus* are detected

Together with *Blastocystis*, ***C. albicans***, is predominant the **gut** of healthy subjects.
Other fungi present are ***S. cerevisiae***, *A. flavus*, *Malassezia pachydermatis*, *M. globosa* and *M. restricta*



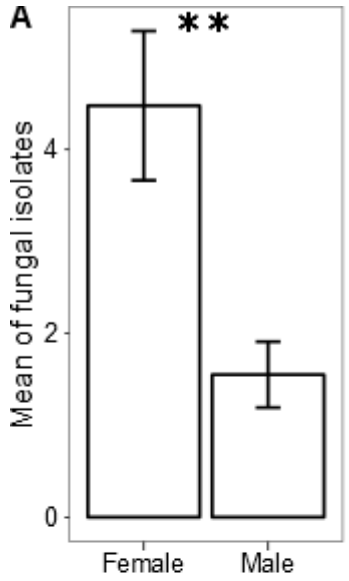
Toe nail showed the great fungal diversity among individuals. ***Malassezia, Aspergillus*** *and* ***Arthrodermataceae*** genera are the most frequent amd abundant together with *Candida, Rhodoturola* and *Criptococcus*.

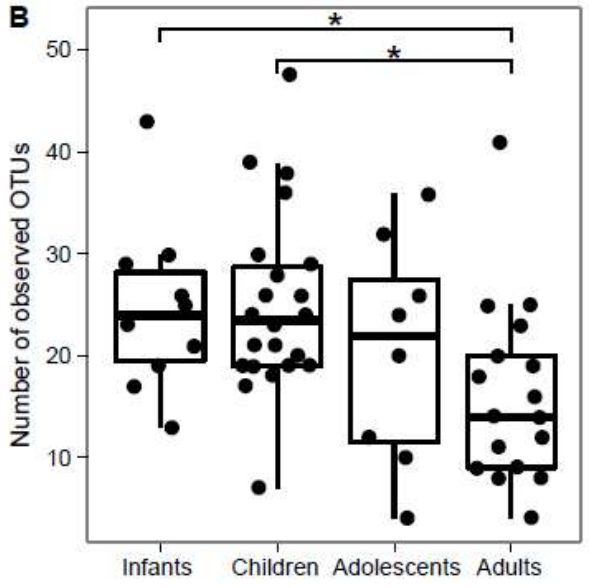
Malassezia, Epicoccum and Aspergillus *are the most frequently isolates from*

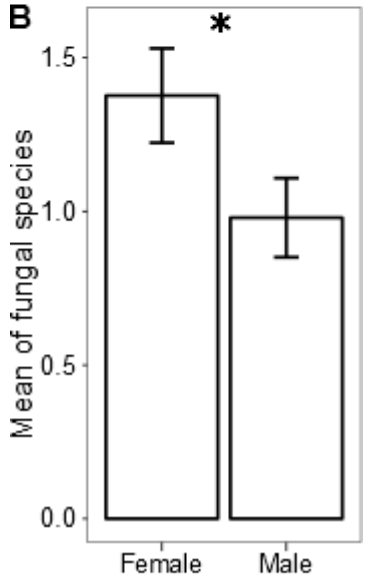
Rizzetto L, De Filippo C, Cavalieri D (2015) Mycobiota: Micro-Eukaryotes Inhabiting Our Body as Commensals or Opportunistic Pathogens. Fungal Genom Biol 4:120.

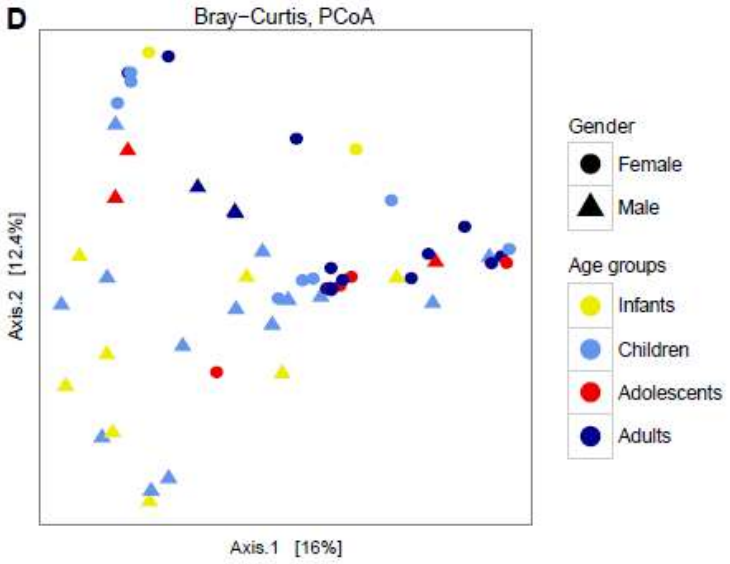
Healthy human gut mycobiota

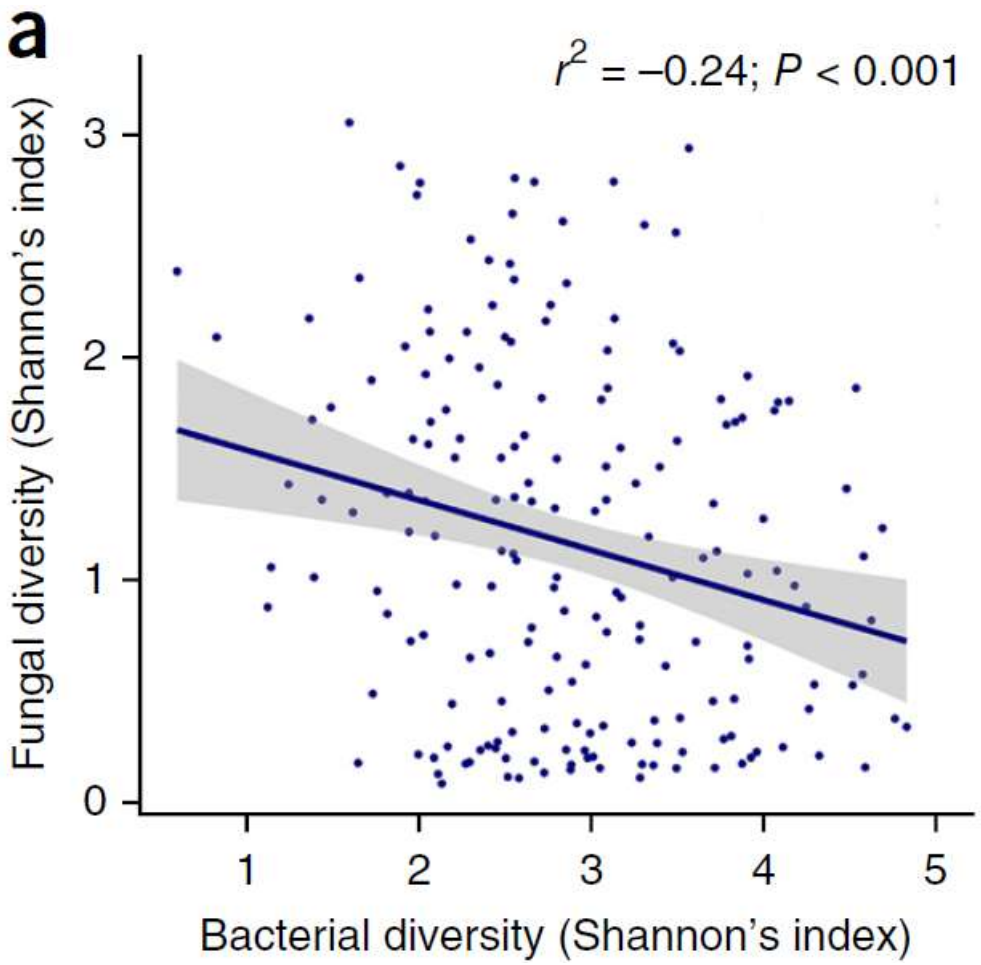
Age and gender shape the human gut mycobiota





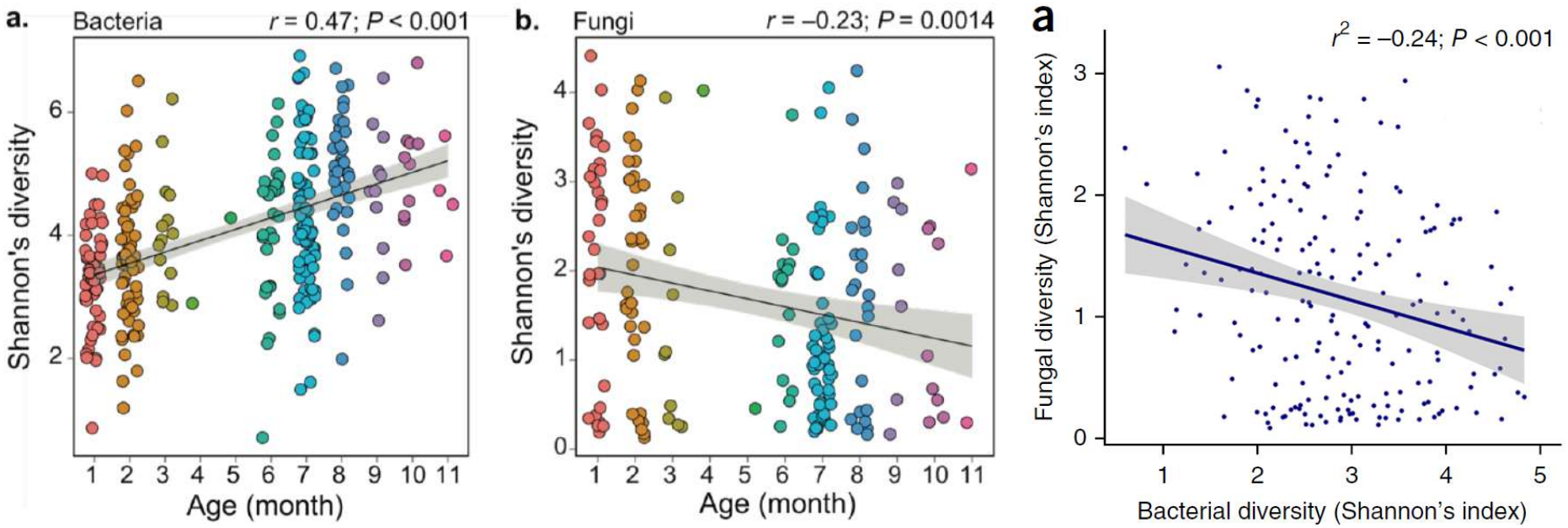






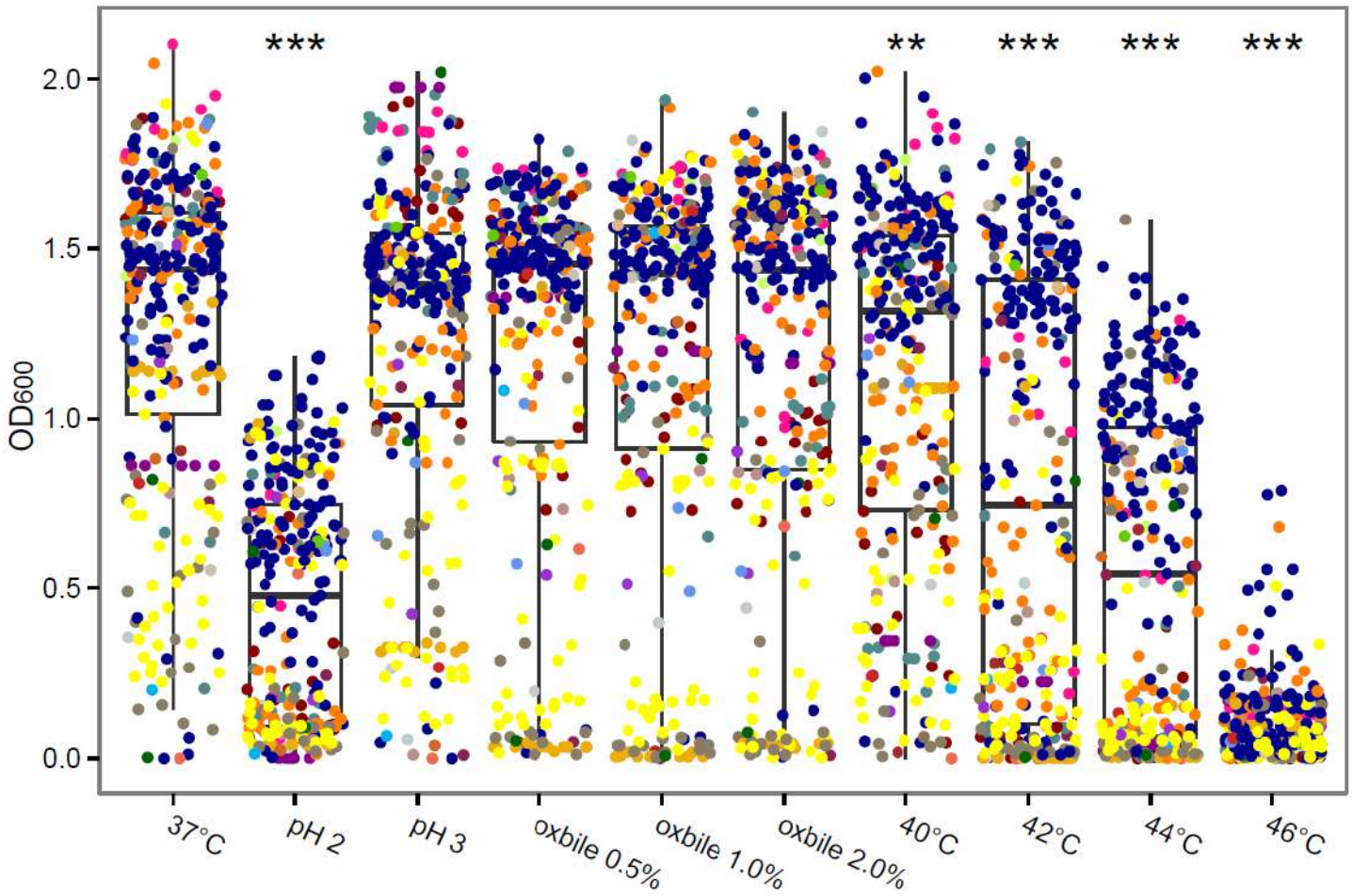
Strati F et al. Frontiers in Microbiology 2016 7:1227

Healthy human gut mycobiota



Bacterial and Fungal Diversity are anticorrelated

Faecal fungal isolates are resistant to GI tract-like stresses



34 different fungal species were isolated showing phenotypic characteristics making them putative commensals of the human GI tract rather than mere passengers, but…

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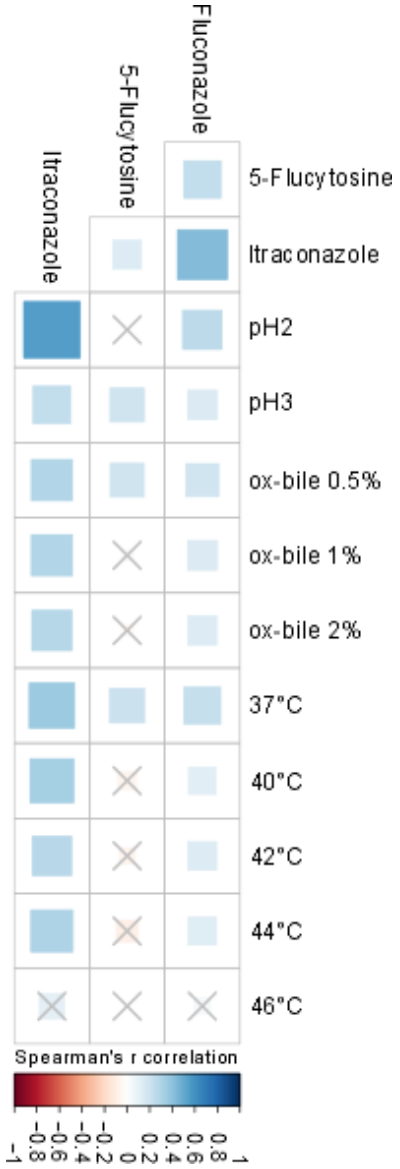
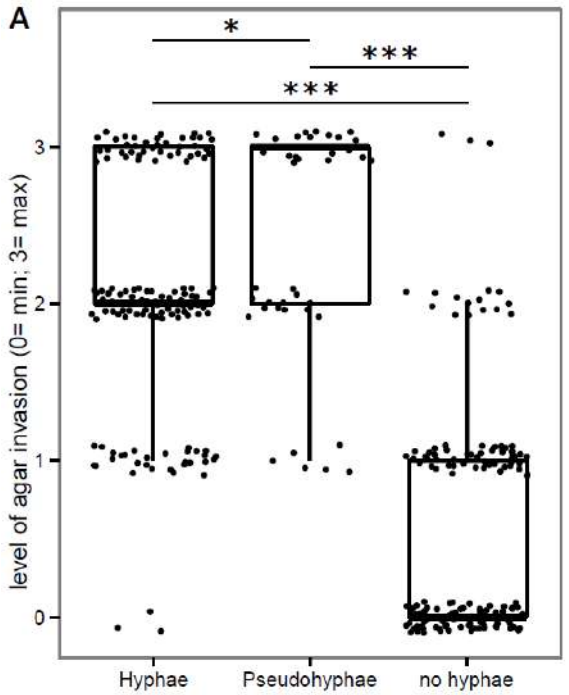
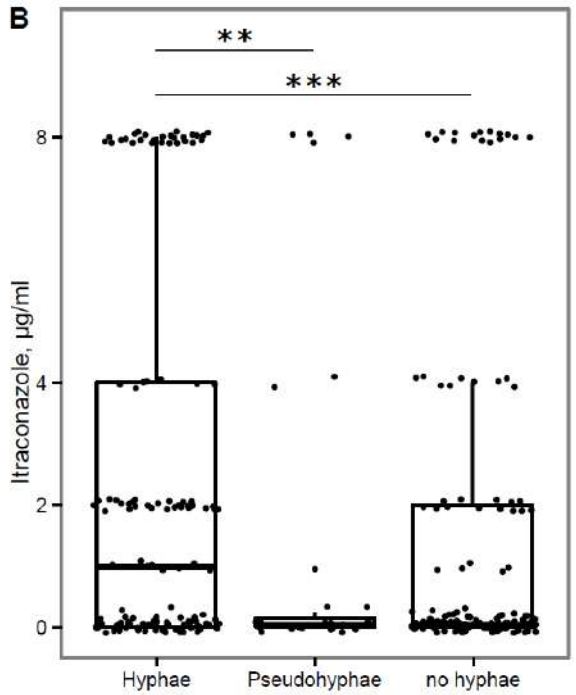
High fungal resistance to azoles







…the high frequency of azoles resistance reported in these isolates could represent an important epidemiological problem not to be underestimated.

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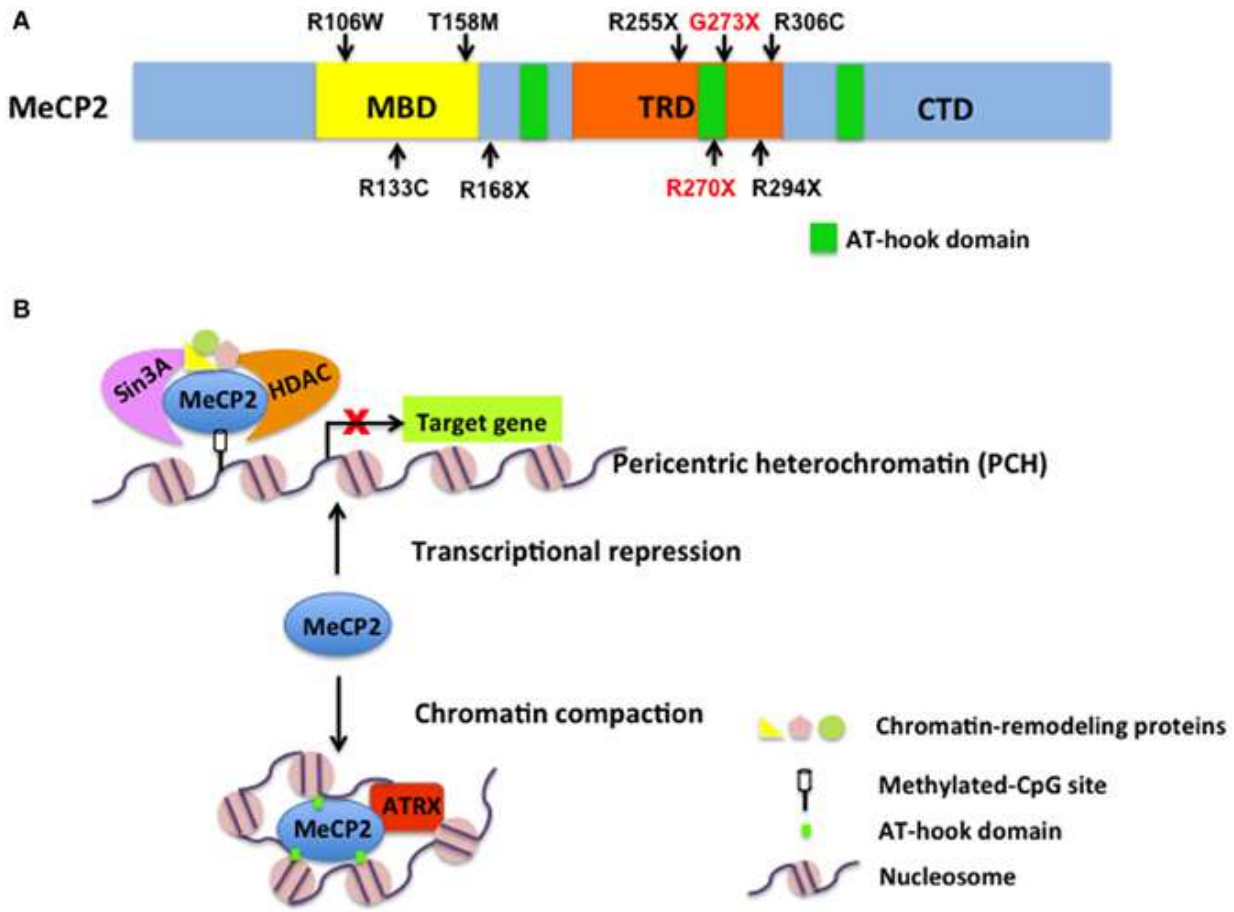
GXM-A model for the interplay between genome and metagenome on the gut brain axis-the Rett syndrome



Strati F et al. Microbiome 2016, 4:41

Gastrointestinal Dysmotility in Rett Syndrome

*Gordon Baikie, †Madhur Ravikumara, ‡Jenny Downs, ‡Nusrat Naseem, ‡Kingsley Wong, §Alan Percy, ||Jane Lane, ¶Batia Weiss, #Carolyn Ellaway, ‡Katherine Bathgate, and ‡Helen Leonard

MeCP2 enforces Foxp3 expression to promote regulatory T cells' resilience to inflammation

Chaoran Li[a,1], Shan Jiang[a,1,2], Si-Qi Liu[a], Erik Lykken[a], Lin-tao Zhao[b], Jose Sevilla[a], Bo Zhu[b], and Qi-Jing Li[a,3]

MeCP2 in the enteric nervous system

G. WAHBA,*,# S. C. SCHOCK,†,# E. CLARIDGE,* M. BETTOLLI,‡ D. GRYNSPAN,‡ P. HUMPHREYS‡ & W. A. STAINES*

*Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada
†Research Institute, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada
‡Departments of Surgery, Pathology and Paediatrics (Neurology), Children's Hospital of Eastern Ontario, Ottawa, ON, Canada

Subclinical Inflammatory Status in Rett Syndrome

Alessio Cortelazzo,[1,2] Claudio De Felice,[3] Roberto Guerranti,[2] Cinzia Signorini,[4] Silvia Leoncini,[1,4] Alessandra Pecorelli,[1,4] Gloria Zollo,[1,4] Claudia Landi,[5] Giuseppe Valacchi,[6,7] Lucia Ciccoli,[4] Luca Bini,[5] and Joussef Hayek[1]

ARTICLE

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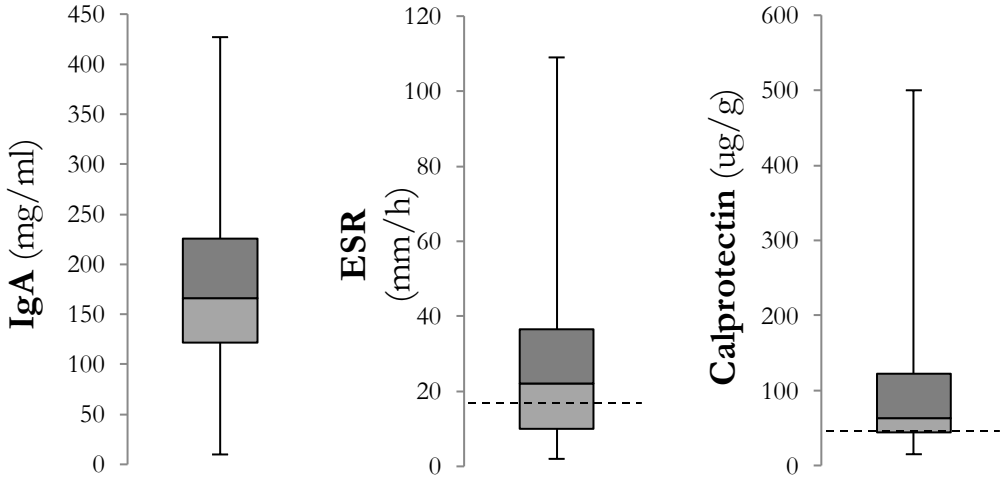
Reduction of aberrant NF-κB signalling ameliorates Rett syndrome phenotypes in *Mecp2*-null mice

Noriyuki Kishi[1,*,†], Jessica L. MacDonald[1,*,†], Julia Ye[1], Bradley J. Molyneaux[1,†], Eiman Azim[1] & Jeffrey D. Macklis[1]

Gastrointestinal and Nutritional Problems Occur Frequently Throughout Life in Girls and Women with Rett Syndrome

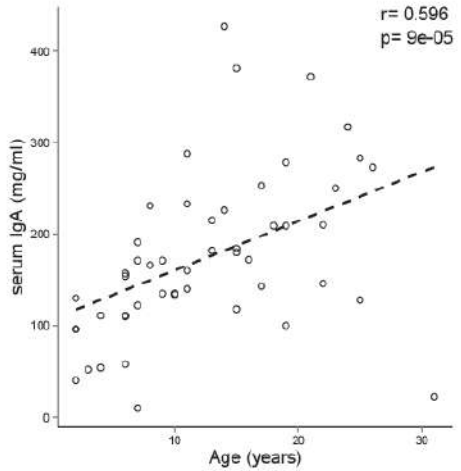
Kathleen J. Motil, M.D., Ph.D.[1], Erwin Caeg, B.B.S.[1], Judy O. Barrish, B.S.N., R.N.[1], Suzanne Geerts, M.S., R.D.[2], Jane B. Lane, B.S.N., R.N.[3], Alan K. Percy, M.D.[4], Fran Annese, L.M.S.W.[5], Lauren McNair, M.S.[5], Steven A. Skinner, M.D.[5], Hye-Seung Lee, Ph.D.[6], Jeffrey L. Neul, M.D., Ph.D.[1], and Daniel G. Glaze, M.D.[1]

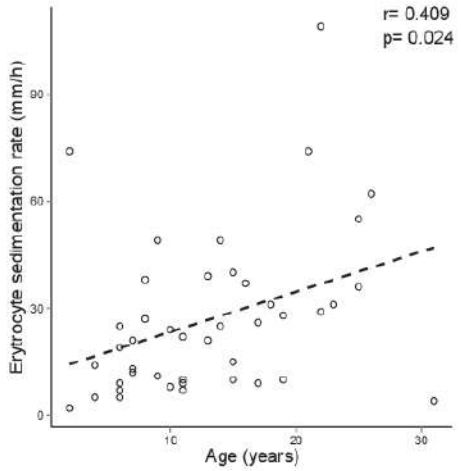
Low-grade intestinal inflammation in RTT

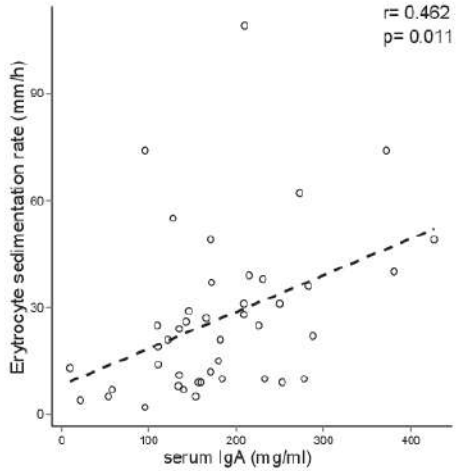


- High levels of ERS*
- High levels of Calprotectin*
- Constipation in 70.8% RTT subjects
- Positive correlation among constipation, IgA, ESR and age

*compared to a healthy population

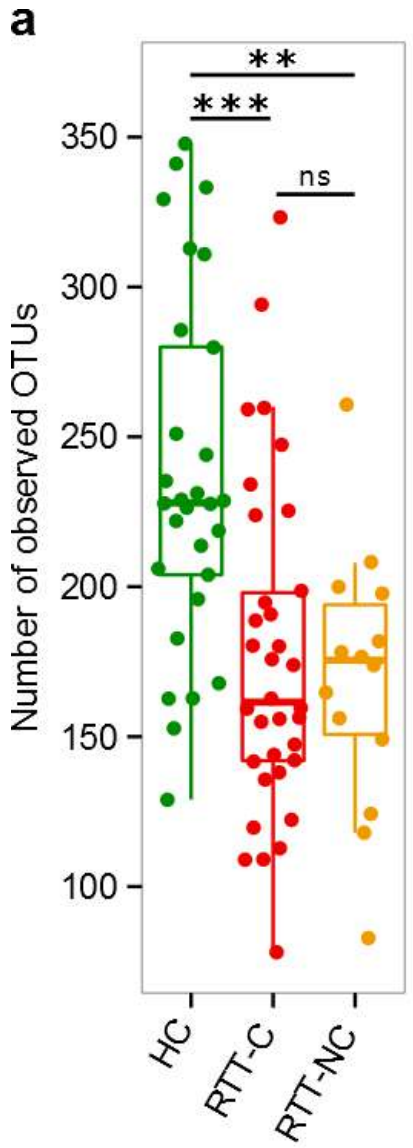


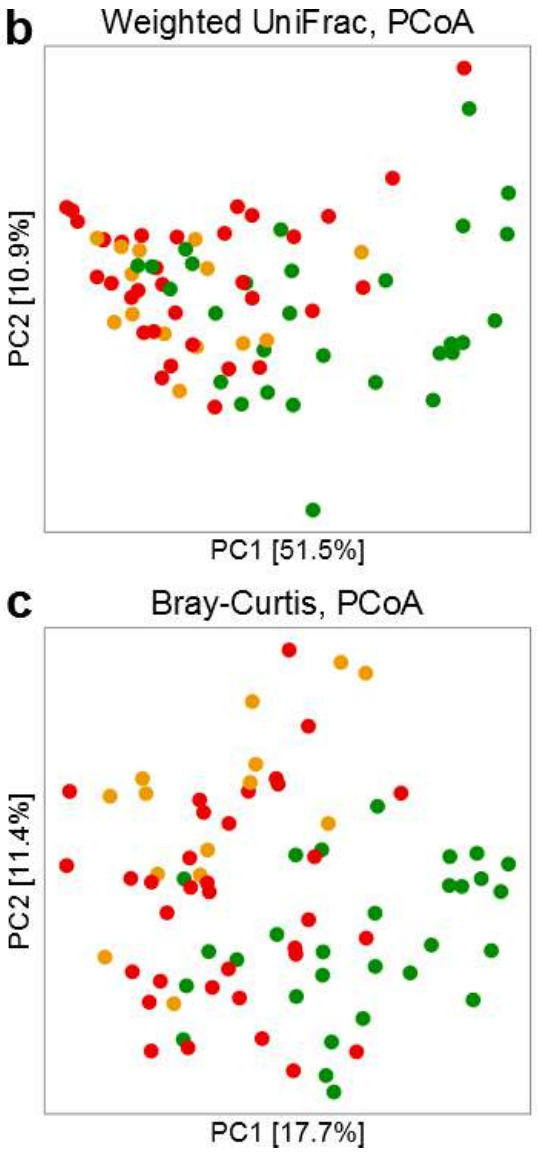




Strati F et al. Microbiome 2016, 4:41

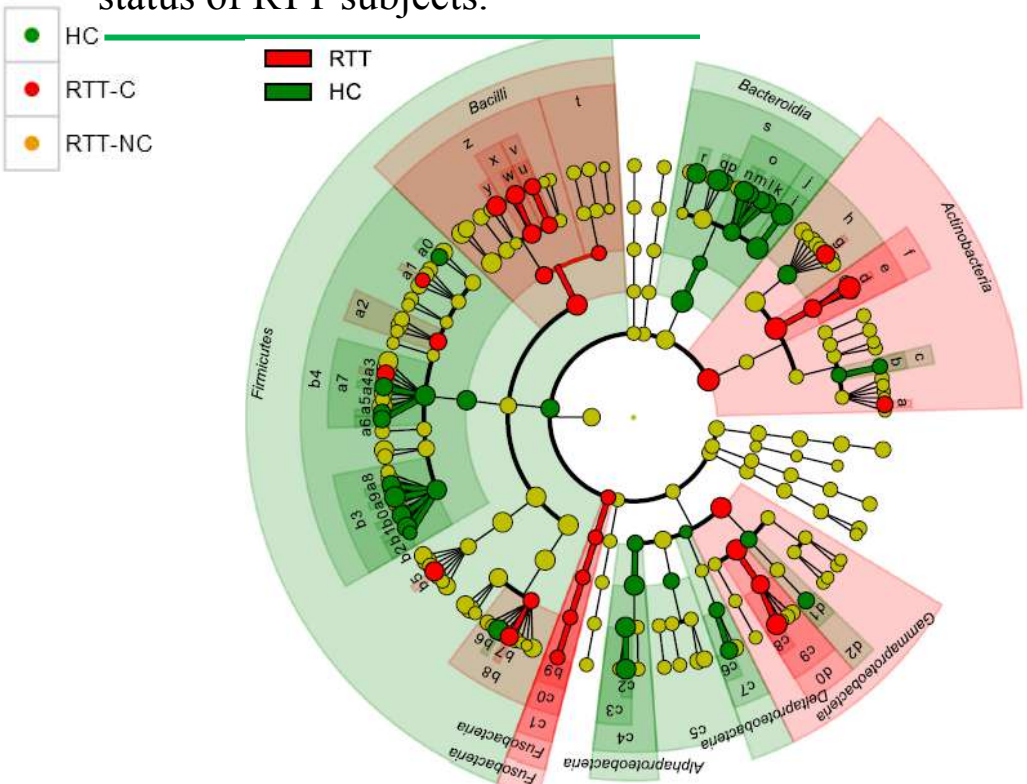
Altered bacterial gut microbiota in RTT





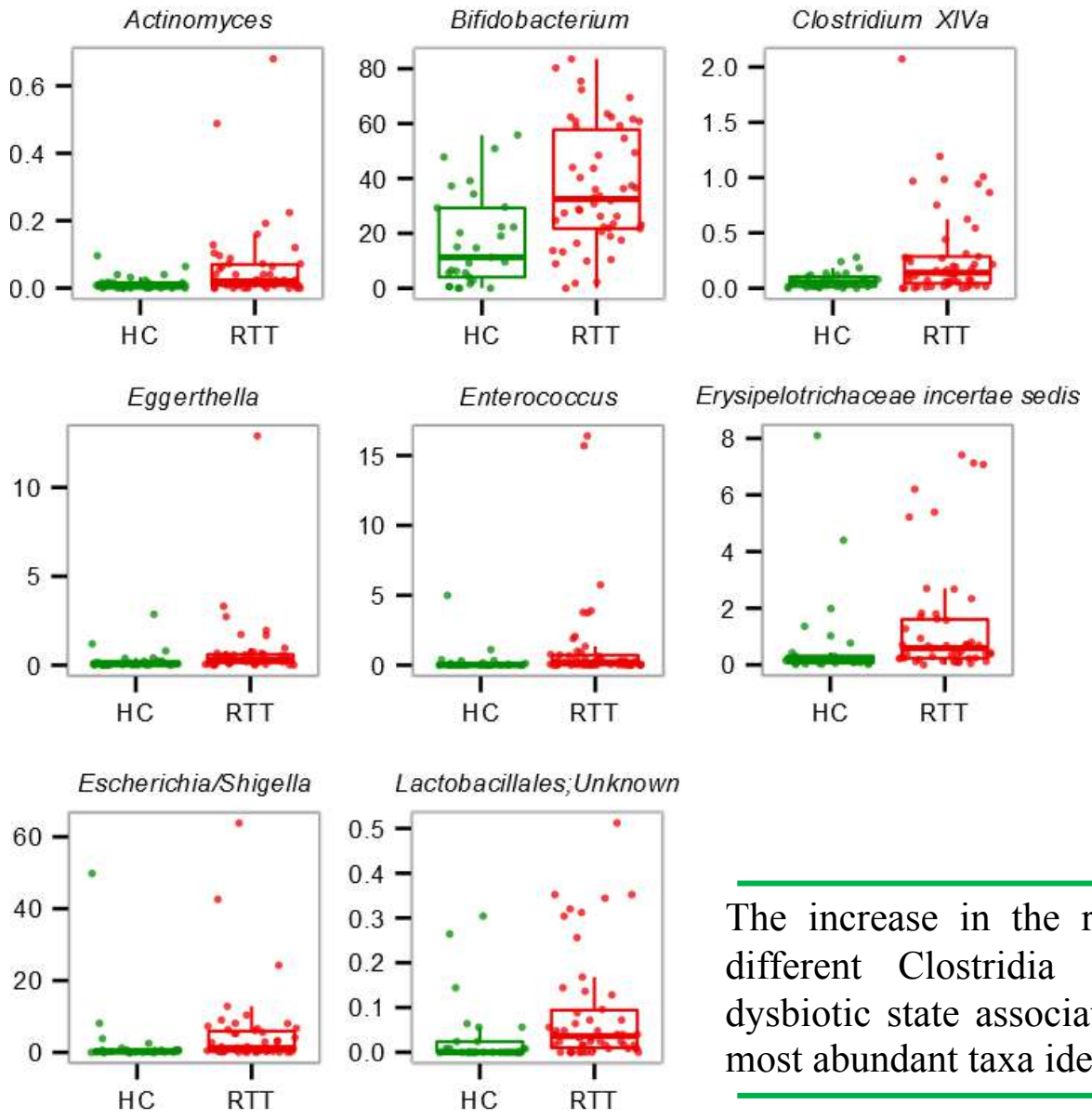
RTT is characterized by a dysbiotic gut microbiota showing an overall reduction of the microbial richness and diversity as well as an altered composition of the microbial community structure.

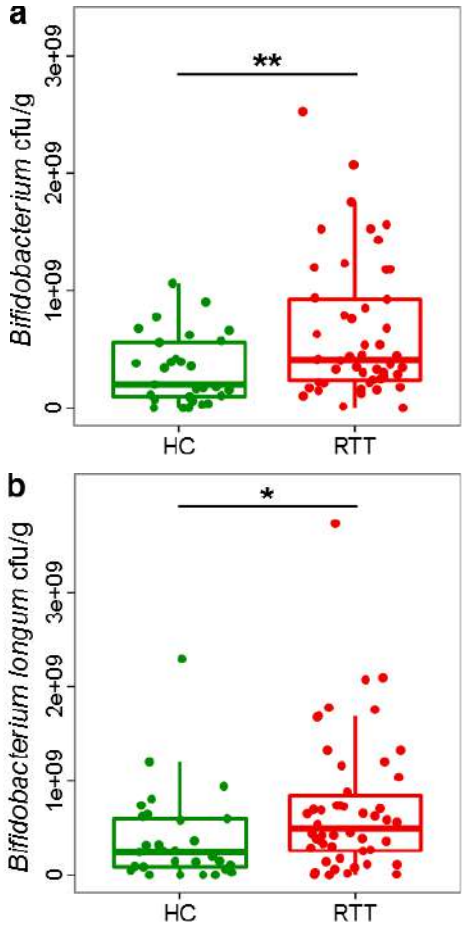
Alterations of the gut microbiota do not depend on the constipation status of RTT subjects.



Strati F et al. Microbiome 2016, 4:41

Bifidobacterium is the hallmark of intestinal dysbiosis in RTT

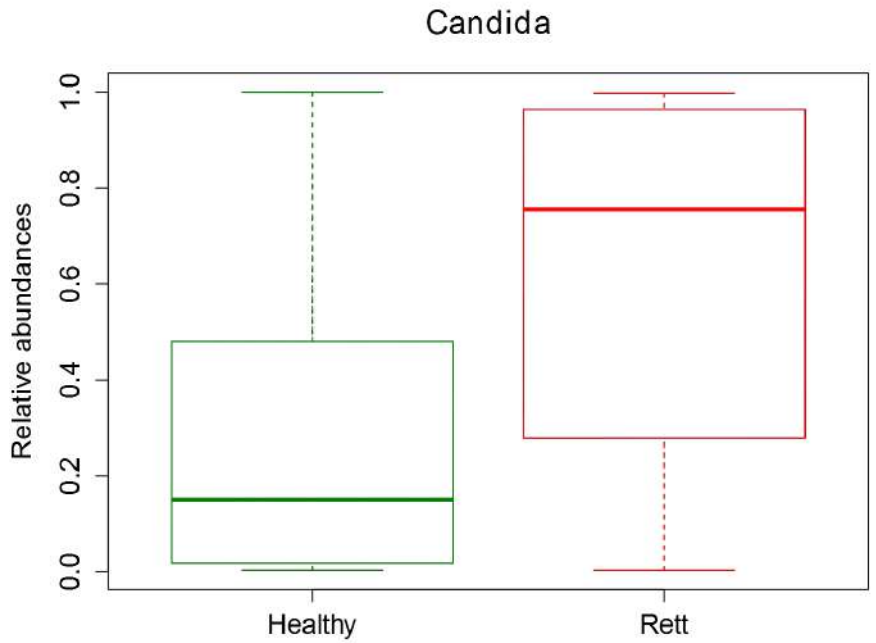


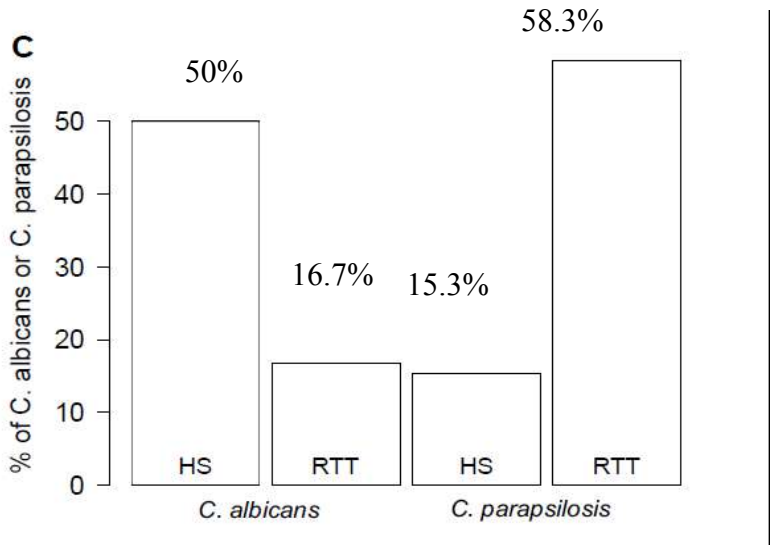


The increase in the relative abundance of *Bifidobacterium*, different Clostridia and *Escherichia/Shigella* drives the dysbiotic state associated with RTT. *Bifidobacterium* was the most abundant taxa identified as confirmed by qPCR analysis.

Strati F et al. Microbiome 2016, 4:41

Rett syndrome associated changes in gut mycobiota population





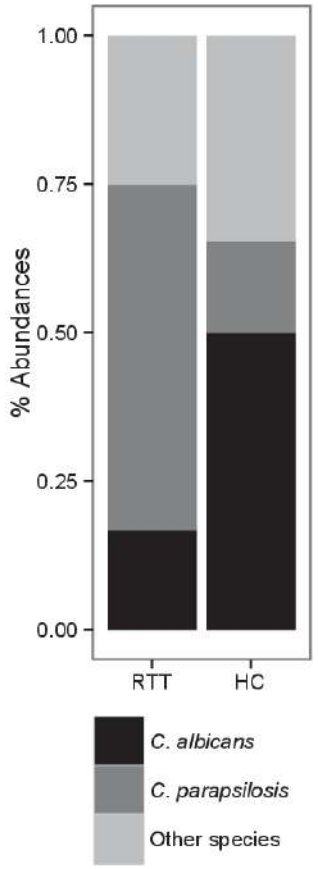
Welch t-test
p=0.0036

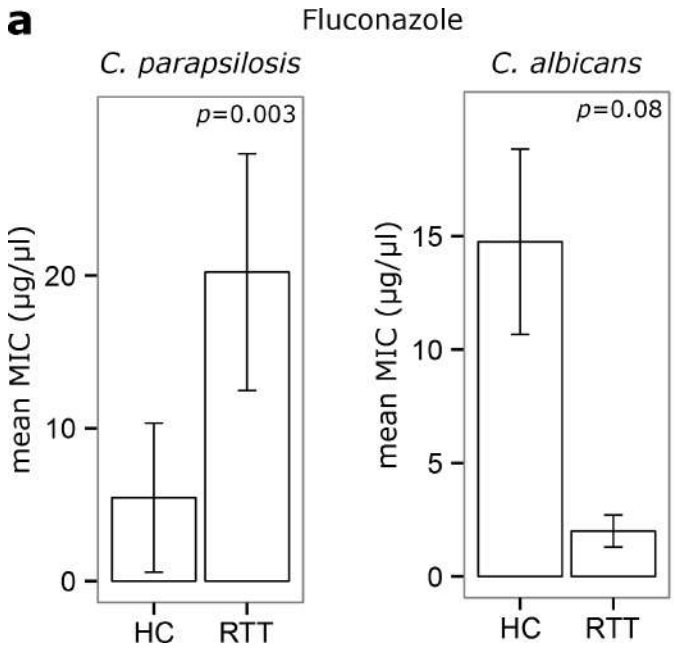
Welch-t statistics of relative abundances at the genus level shown ***Candida*** as significantly more abundant in Rett syndrome patients compared to Healthy Subjects

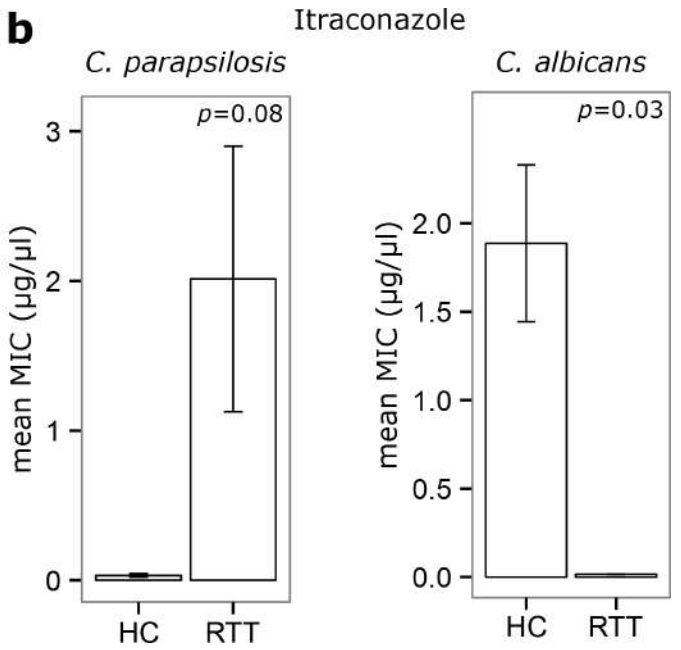
Strati F, Cavalieri D, Albanese D, De Felice C, Donati C, Hayek J, Jousson O, Leoncini S, Pindo M, Renzi D, Rizzetto L, Stefanini I, Calabrò A, De Filippo C. Altered gut microbiota in Rett syndrome. Microbiome. 2016 Jul 30;4(1):41

Strati F et al. Microbiome 2016, 4:41

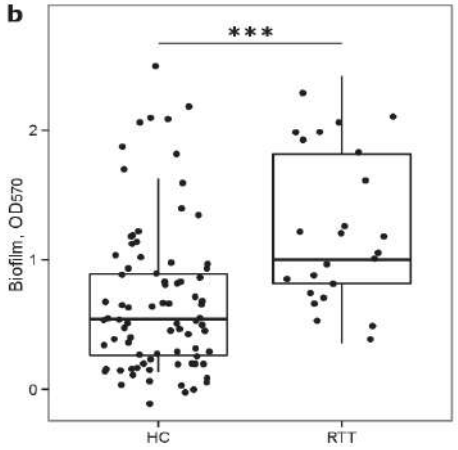
Candida parapsilosis from RTT hold potential virulent traits...





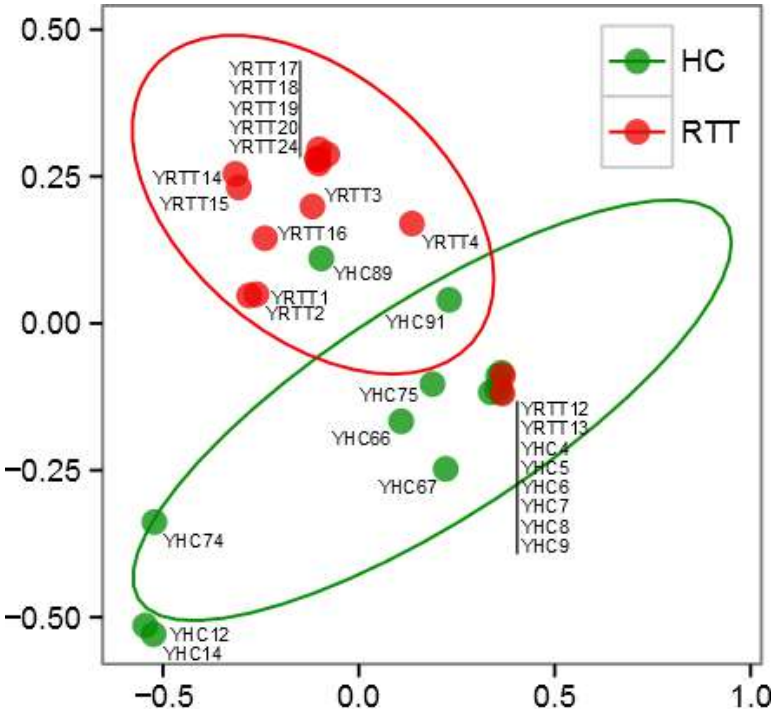


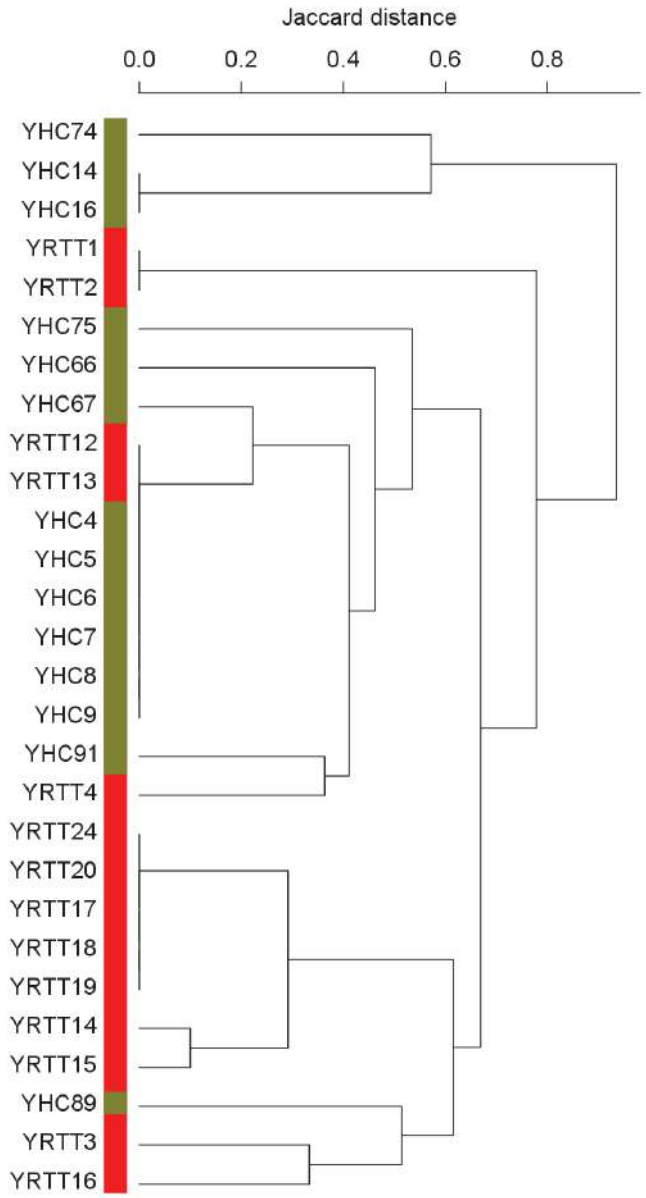
C. parapsilosis was the most abundant yeast species in RTT subjects, characterized by high levels of resistance to azoles, making it a difficult target in case of fungal infections in these subjects.



FONDAZIONE EDMUND MACH

RTT C. ps are genetically unrelated to HC Cp isolates

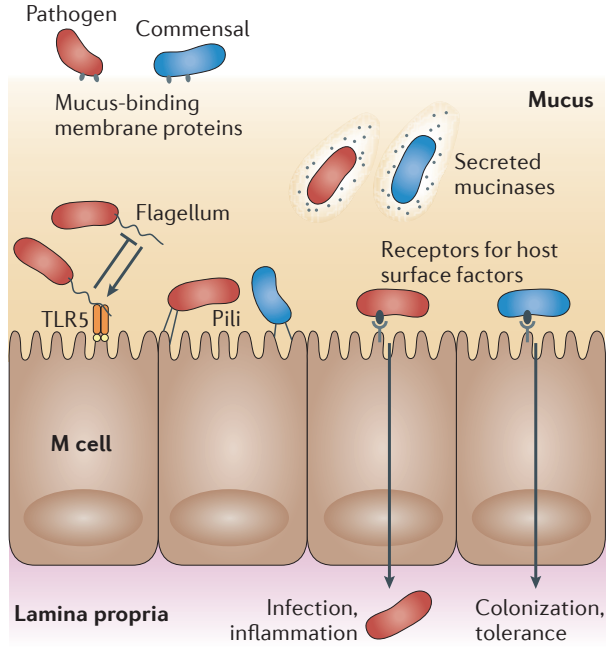


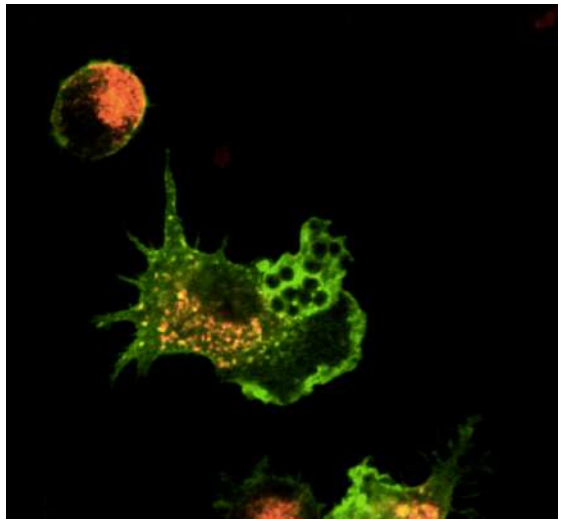


RTT's *C. parapsilosis* isolates were genetically unrelated to HC's *C. parapsilosis* isolates as measured by UPMGA hierarchical clustering analysis from RAPD genotyping.

Fungi are major immune modulator

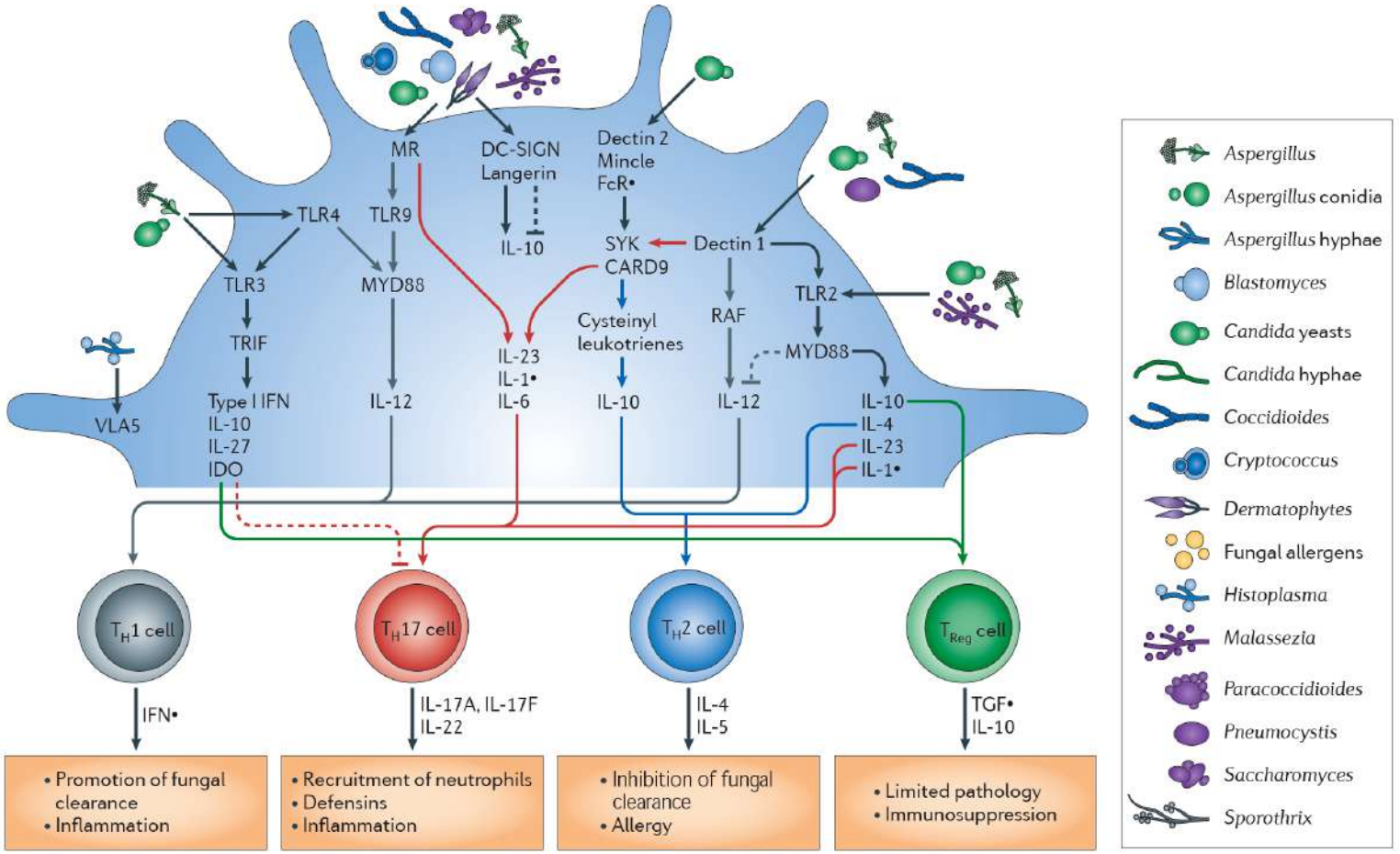
Yeast
&
me





Rizzetto L, De Filippo C, Cavalieri D.
Richness and diversity of mammalian fungal communities shape innate and adaptive immunity in health and disease.
Eur J Immunol. 2014 Sep 25.

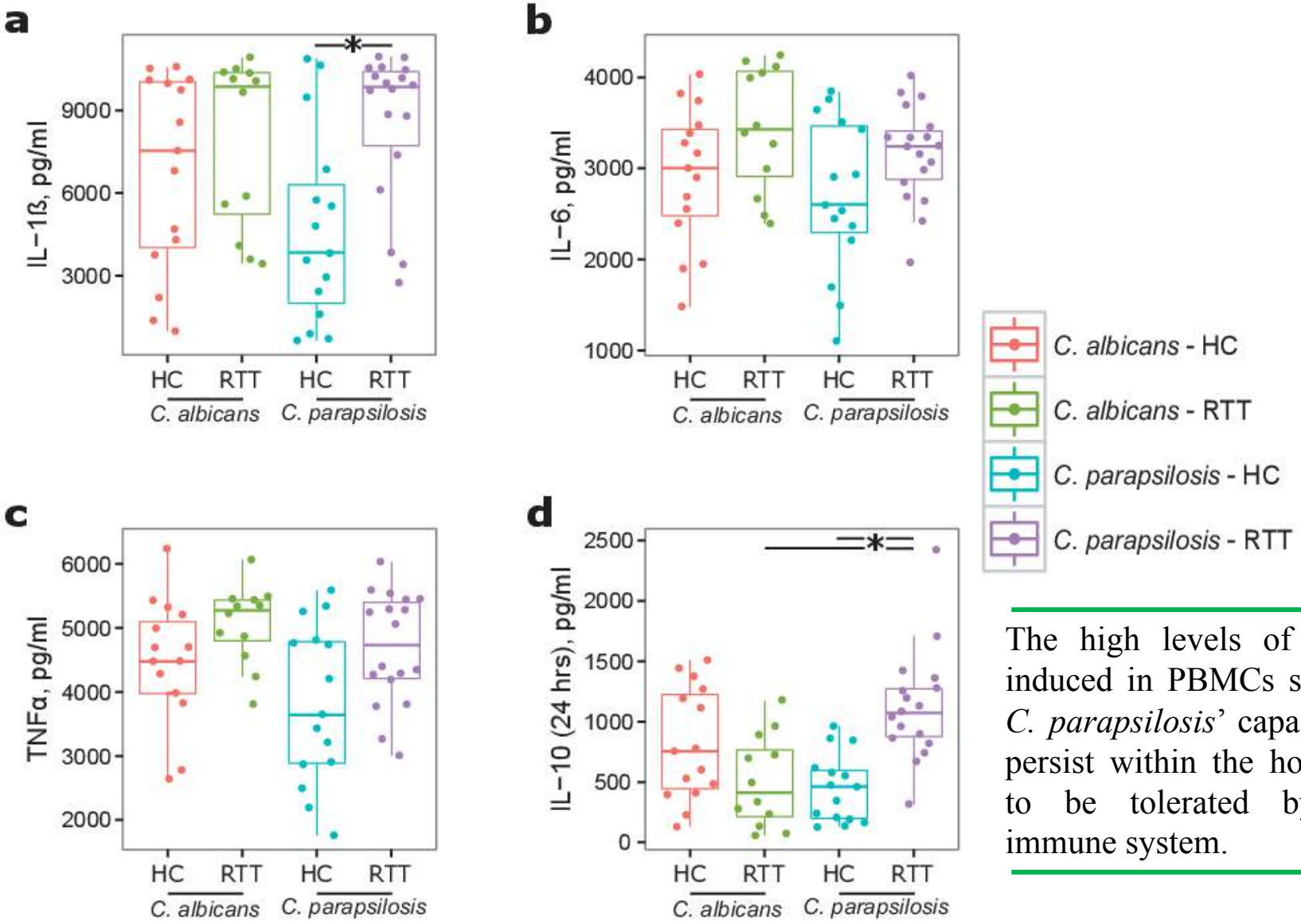
Specific PRRs lead the host immune surveillance and determine subsequent fungal antigen processing and antigen presentation



The highly dynamic spatio-temporal signal integration implies that coordinated regulation of RNA levels corresponds to precise cytokine expression changes.

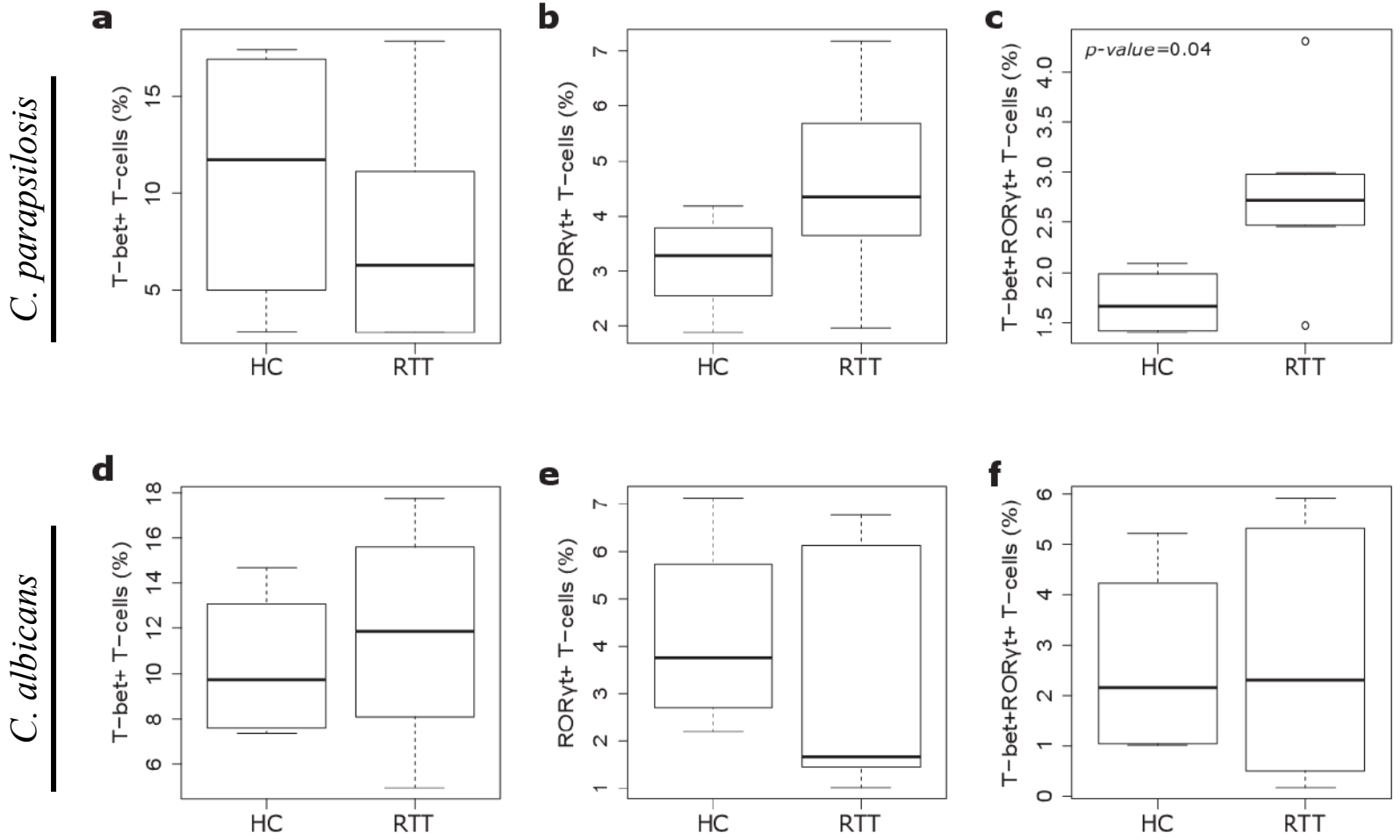
Figure adapted from Romani L, Nature, 2011

...C. parapsilosis capacity to persist within the host favors inflammation



The high levels of IL-10 induced in PBMCs suggest *C. parapsilosis*' capacity to persist within the host and to be tolerated by the immune system.

...and capacity to persist within the host favoring inflammation



RTT *C. parapsilosis* isolates induced a higher proportion of a mixed Th1/Th17 cells population compared to HC *C. parapsilosis* isolates known to be involved in pro-inflammatory responses possibly resulting in adaptive immunity against the commensal microbiota.

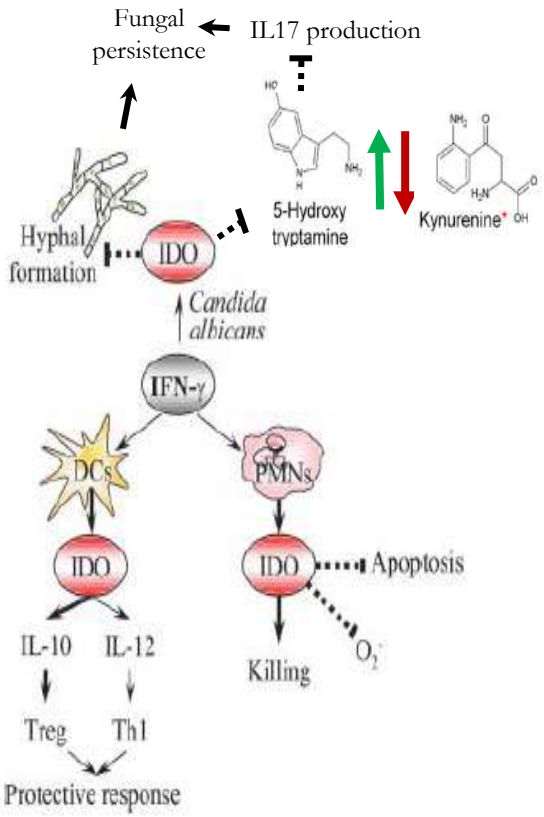
Central role of tryptophan metabolism in host/yeast interaction

Candida albicans Dampens Host Defense by Downregulating IL-17 Production

Shih-Chin Cheng, Frank van de Veerdonk, Sanne Smeekens, Leo A. B. Joosten, Jos W. M. van der Meer, Bart-Jan Kullberg and Mihai G. Netea

A Crucial Role for Tryptophan Catabolism at the Host/ _Candida albicans_ Interface

Silvia Bozza, Francesca Fallarino, Lucia Pitzurra, Teresa Zelante, Claudia Montagnoli, Silvia Bellocchio, Paolo Mosci, Carmine Vacca, Paolo Puccetti and Luigina Romani

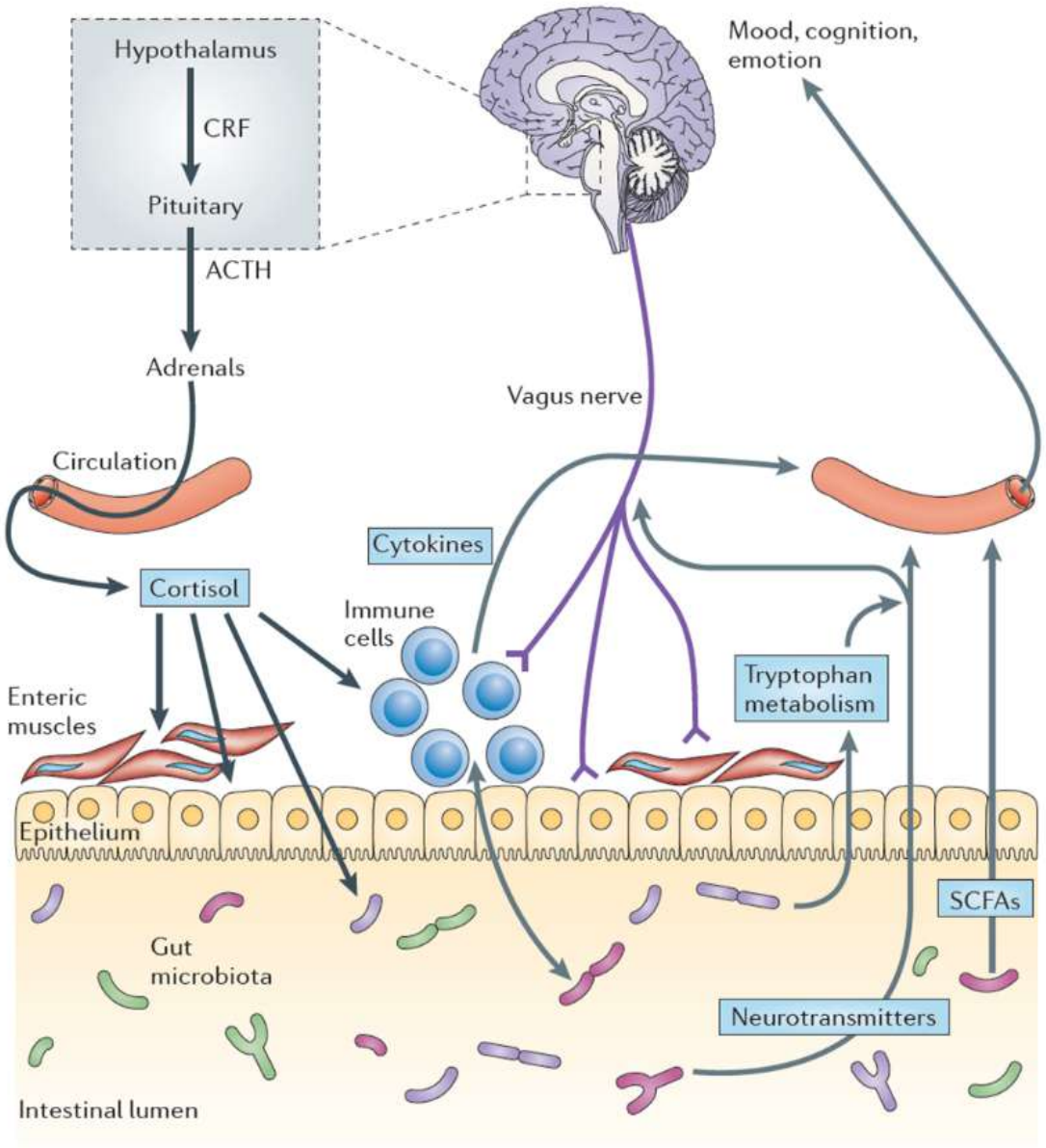


Tryptophan Catabolites from Microbiota Engage Aryl Hydrocarbon Receptor and Balance Mucosal Reactivity via Interleukin-22

Teresa Zelante,[1] Rossana G. Iannitti,[1] Cristina Cunha,[1] Antonella De Luca,[1] Gloria Giovannini,[1] Giuseppe Pieraccini,[2] Riccardo Zecchi,[2] Carmen D'Angelo,[1] Cristina Massi-Benedetti,[1] Francesca Fallarino,[1] Agostinho Carvalho,[1] Paolo Puccetti,[1,3] and Luigina Romani[1,3,*]

- _Candida albicans_ infection upregulated IDO expression and kynurenine production at the sites of infection (Bozza et al., 2005).
- _C. albicans_ shifts IDO's activity towards the formation of hydroxytryptophan metabolites that inhibit IL17 production and towards the induction of Treg cells which favor fungal persistence (De Luca et al., 2007; Cheng et al., 2010).
- IDO's blokade promote yeast to hyphal transition

The interplay between the intestinal microbiota and the CNS

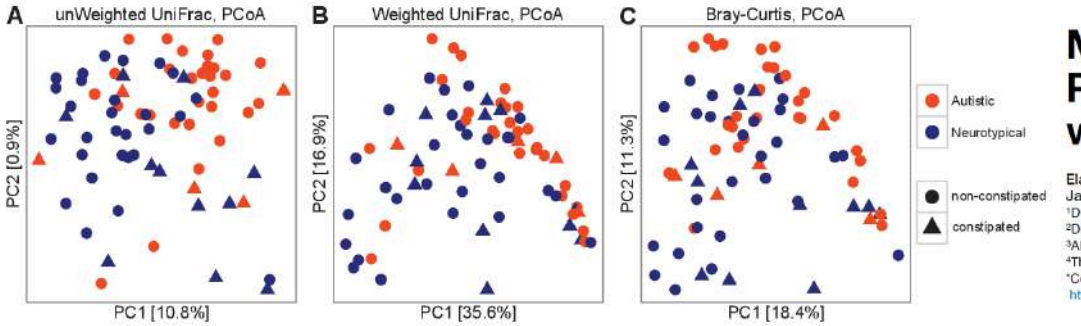


Bidirectional communication gut microbiota-CNS

- the autonomous nervous system (ANS), in particular the enteric nervous system (ENS) and the vagal nerves
- the hypothalamic-pituitary-adrenal (HPA) axis
- tryptophan metabolism and other bacterial metabolites
- the immune system

Cryan and Dinan, Nature Rev. Neurosci. 13:701-712, 2012

Other examples of involvement gut microbiota in CNS disorders



Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders

Elaine Y. Hsiao,[1,2,*] Sara W. McBride,[1] Sophia Hsien,[1] Gil Sharon,[1] Embriette R. Hyde,[3] Tyler McCue,[3] Julian A. Codelli,[2] Janet Chow,[1] Sarah E. Reisman,[2] Joseph F. Petrosino,[3] Paul H. Patterson,[1,4,*] and Sarkis K. Mazmanian[1,4,*]
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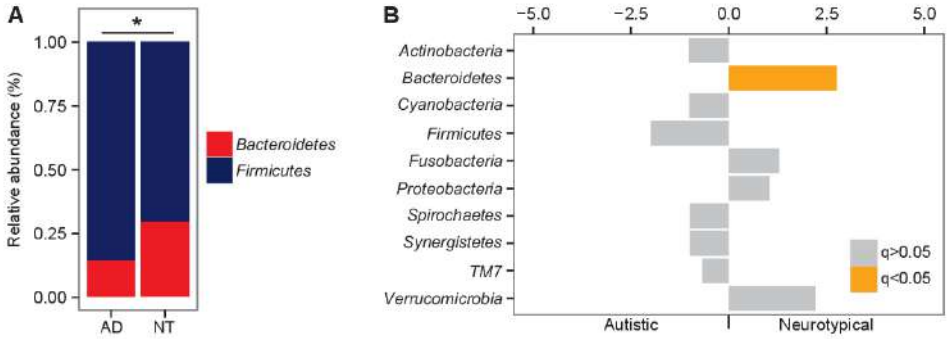
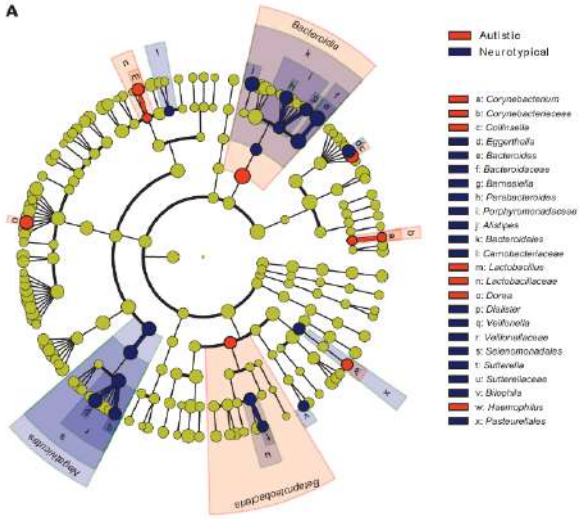
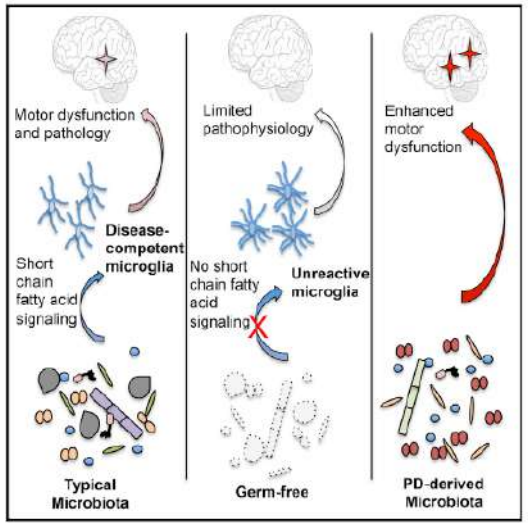
[2]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA
[3]Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine, Houston, TX 77030, USA
[4]These authors contributed equally to this work
*Correspondence: ehsiao@caltech.edu (E.Y.H.), php@caltech.edu (P.H.P.), sarkis@caltech.edu (S.K.M.)
http://dx.doi.org/10.1016/j.cell.2013.11.024





Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease

Graphical Abstract



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In Brief
Signals from gut microbes are required for the neuroinflammatory responses as well as hallmark gastrointestinal and α-synuclein-dependent motor deficits in a model of Parkinson's disease.

Alterations of the gut microbiota has been observed in a wide variety of neurological disorders: Multiple Sclerosis, Alzheimer, Parkinson, **Autism Spectrum Disorders**.

DOI 10.1186/s40168-017-0242-1

Microbiome

RESEARCH Open Access

New evidences on the altered gut microbiota in autism spectrum disorders

Francesco Strati[1,2], Duccio Cavalieri[3], Davide Albanese[1], Claudio De Felice[4], Claudio Donati[1], Joussef Hayek[5,6], Olivier Jousson[2], Silvia Leoncini[5], Daniela Renzi[7], Antonio Calabrò[7] and Carlotta De Filippo[8*]

Abstract

Background: Autism spectrum disorders (ASDs) are neurodevelopmental conditions characterized by social and behavioural impairments. In addition to neurological symptoms, ASD subjects frequently suffer from gastrointestinal abnormalities, thus implying a role of the gut microbiota in ASD gastrointestinal pathophysiology.

Results: Here, we characterized the bacterial and fungal gut microbiota in a cohort of autistic individuals demonstrating the presence of an altered microbial community structure. A fraction of 90% of the autistic subjects were classified as severe ASDs. We found a significant increase in the *Firmicutes/Bacteroidetes* ratio in autistic subjects due to a reduction of the *Bacteroidetes* relative abundance. At the genus level, we observed a decrease in the relative abundance of *Alistipes*, *Bilophila*, *Dialister*, *Parabacteroides*, and *Veillonella* in the ASD cohort, while *Collinsella*, *Corynebacterium*, *Dorea*, and *Lactobacillus* were significantly increased. Constipation has been then associated with different bacterial patterns in autistic and neurotypical subjects, with constipated autistic individuals characterized by high levels of bacterial taxa belonging to *Escherichia/Shigella* and *Clostridium cluster XVIII*. We also observed that the relative abundance of the fungal genus *Candida* was more than double in the autistic than neurotypical subjects, yet due to a larger dispersion of values, this difference was only partially significant.

Conclusions: The finding that, besides the bacterial gut microbiota, also the gut mycobiota contributes to the alteration of the intestinal microbial community structure in ASDs opens the possibility for new potential intervention strategies aimed at the relief of gastrointestinal symptoms in ASDs.

Keywords: Autism spectrum disorders, Gut microbiota, Mycobiota, Constipation, Metataxonomy

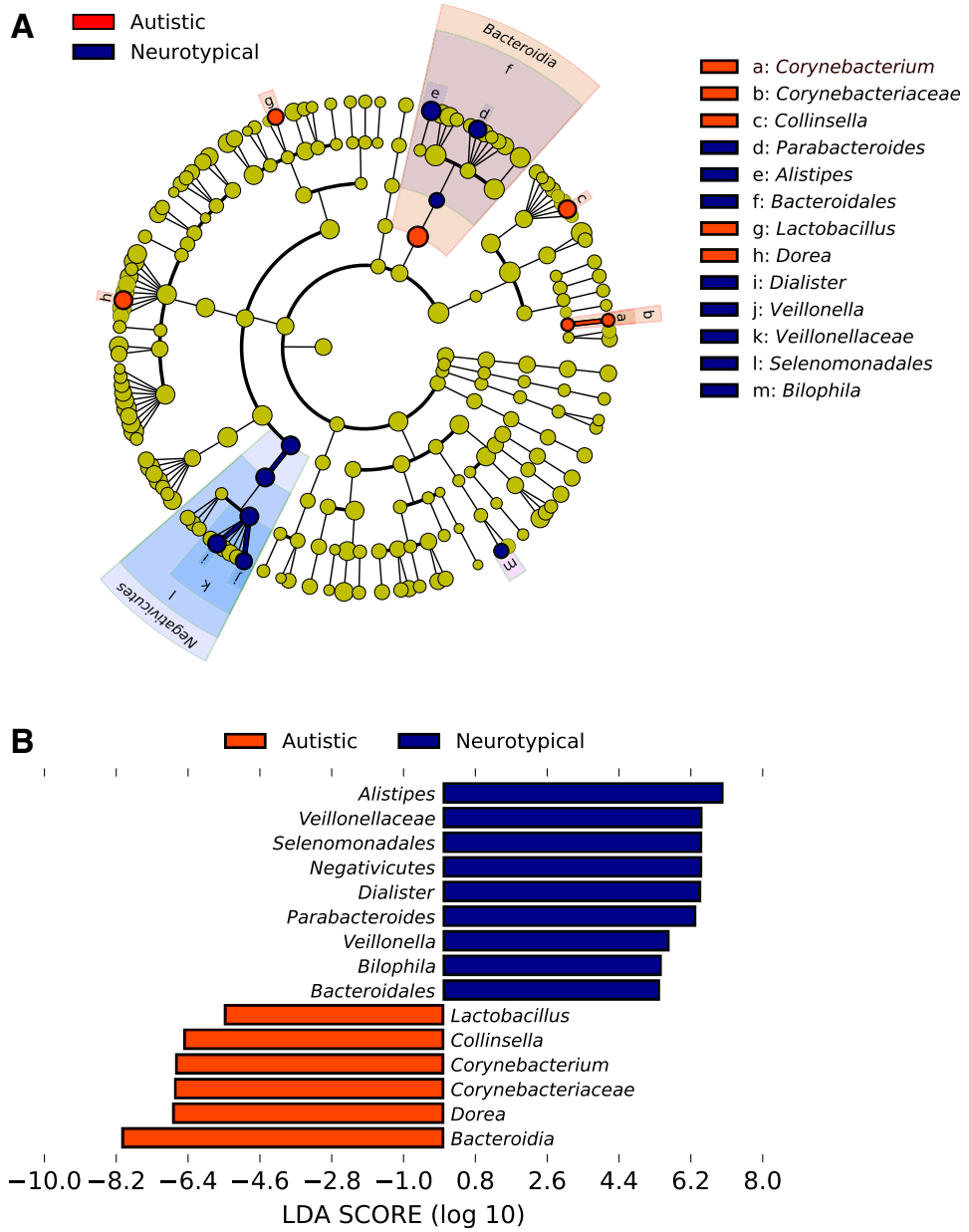


Fig. 3 a Cladograms generated by LEfSe indicating differences in the bacterial taxa between autistic (AD) and neurotypical (NT) subjects. Nodes in *orange* indicate taxa that were enriched in AD compared to those in NT, while nodes in *blue* indicate taxa that were enriched in NT compared to those in AD. **b** LDA scores for the bacterial taxa differentially abundant between autistic (AD) and neurotypical (NT) subjects. Positive and negative LDA scores indicate the bacterial taxa enriched in NT and AD subjects, respectively. Only the taxa having a $p < 0.01$ (Wilcoxon rank-sum test) and LDA >2.0 are shown in the figure legend

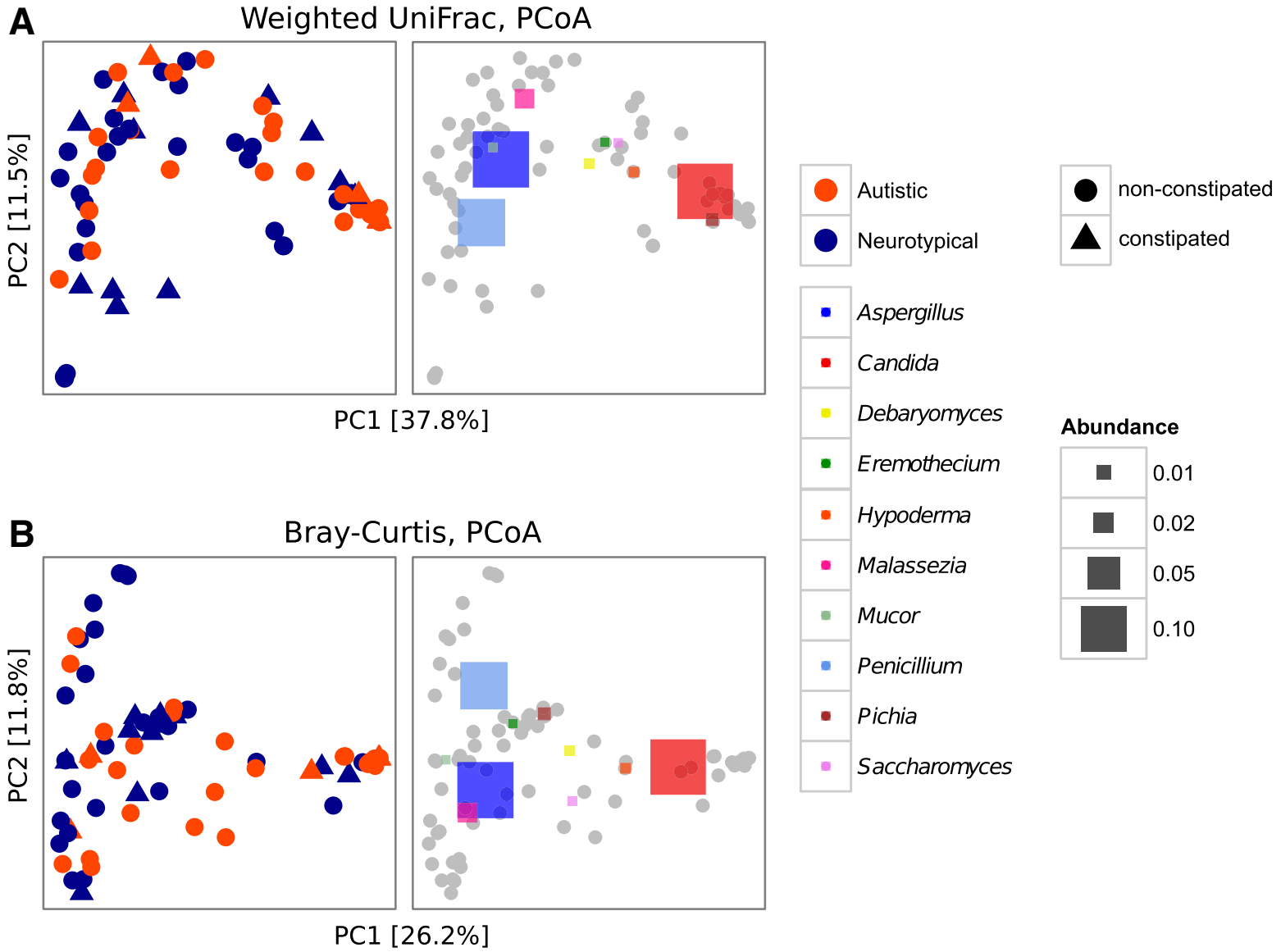


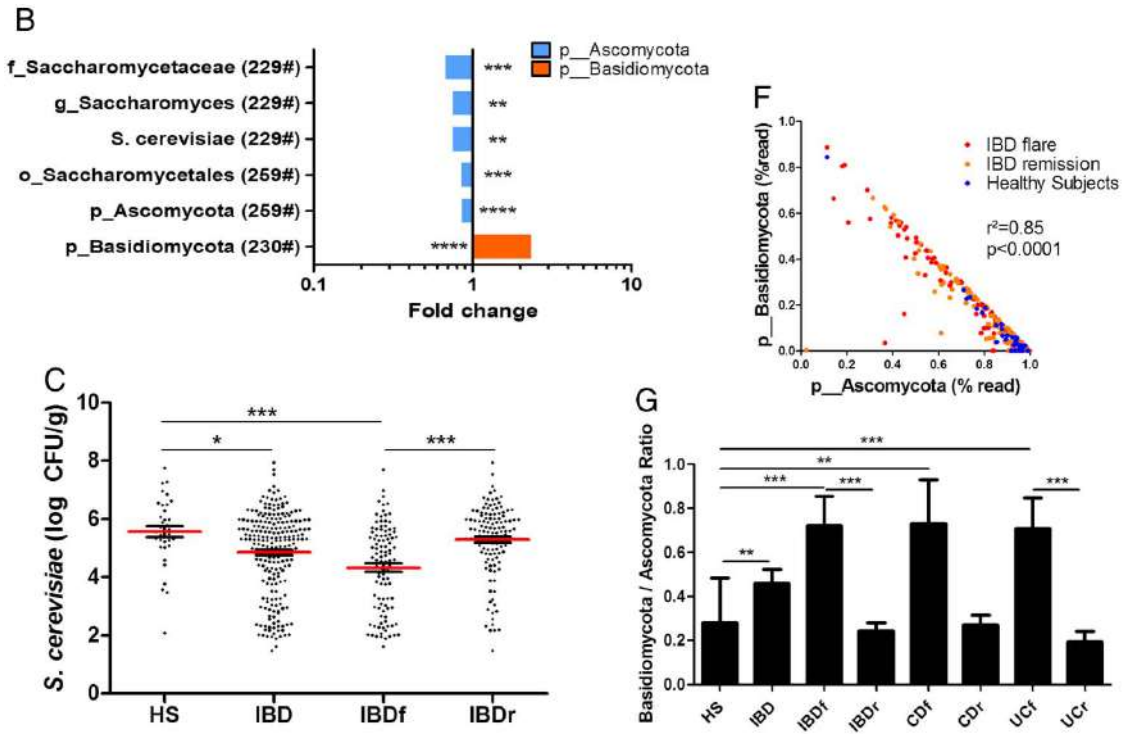
Fig. 5 PCoAs of fungal beta diversity based on **a** weighted UniFrac distance and **b** Bray-Curtis dissimilarity. The right panel of the graphs **a** and **b** shows the same PCoA coordinates with the most abundant OTUs superimposed as *coloured squares*, with the size being proportional to the mean relative abundance of the taxon across all samples (*grey dots*). Autistic and neurotypical subjects are colored in *orange* and *blue*, respectively. The constipation status of the subjects is indicated according to different shapes, *circles* for non-constipated and *triangles* for constipated individuals

Taxa	HS (mean%)	ASD (mean%)
Bifidobacterium	21,77406	24,89999
Bacteroides	12,66584	5,654461
Faecalibacterium	6,295548	10,2981
Unknown	6,408043	6,3966
Blautia	4,079237	6,253344
Ruminococcus	3,900612	3,448255
Clostridium XI	3,096802	3,544552
Streptococcus	4,649735	1,723385
Gemmiger	3,043601	3,01061
Lachnospiracea	2,418118	3,041075
Unknown	2,46166	2,392112
Escherichia/Shigella	3,183142	1,470606
Alistipes	3,165904	1,284848
Anaerostipes	1,914641	2,445164
Clostridium XVIII	1,229121	2,464483
Dorea	1,102211	2,116448
Collinsella	0,737681	2,421239
Clostridium sensu stricto	1,133419	1,701391
Erysipelotrichaceae	0,459639	1,622035

Taxa	ASD (Mean%)	HS (Mean%)
Aspergillus	24,22489	28,02919
Candida	37,70064	14,16352
Penicillium	13,24136	23,48199
Fungi_unidentified_1_1	4,886515	4,35044
Blastocystis	0	7,741706
Malassezia	3,047026	3,29289
Tremellomycetes_unidentified_1	3,103397	2,997759
Unknown	2,038273	1,634148
Pichia	2,762201	0,043723
Basidiomycota_unidentified_1_1	0,050438	2,617916
Ascomycota_unidentified_1_1	0,364931	2,011259
Hypoderma	1,91663	0
Debaryomyces	0,287791	1,418265
Saccharomyces	1,269841	0,273269
Mucor	0,344163	1,123135
Dothideomycetes_unidentified_1	0,688325	0,696836
Eremothecium	0	1,112204
Xeromyces	0,094941	0,617588
Aureobasidium	0,504376	0,199486
Davidiella	0,367898	0,245942
Cyberlindnera	0,311526	0,21315
Trichosporon	0,005934	0,472755
Podosphaera	0,474707	0
Mucoraceae_unidentified	0,130544	0,314259
Thermomyces	0,418336	0,019129

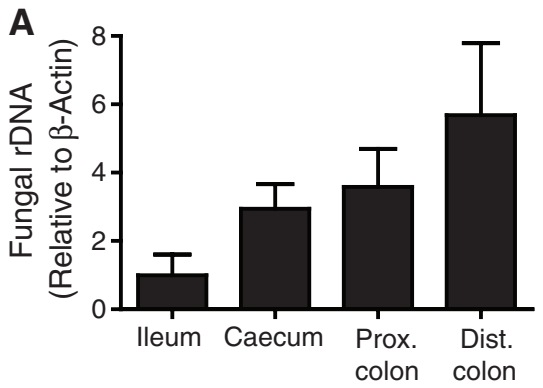
Candida

The gut microbiota in inflammatory bowel diseases



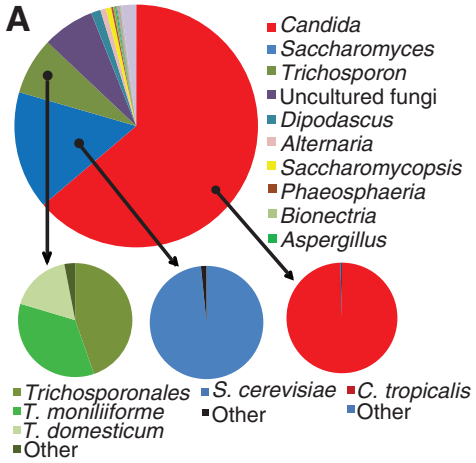
Sokol H et al. Gut 2016;0:1–10

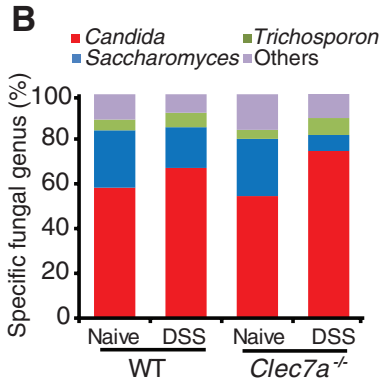
Fungal gut microbiota



Intestinal fungi in WT and Clec7a$^{-/-}$ mice
before and after dextran sodium sulfate (DSS).

In Clec7a$^{-/-}$ mice treated with DSS an abundance of *Candida* and a decrease of *Saccharomyces* species were observed.





Iliev ID et al. Science 2012, 336(6086):1314-1317

Fungal metagenome analysis of CD patients

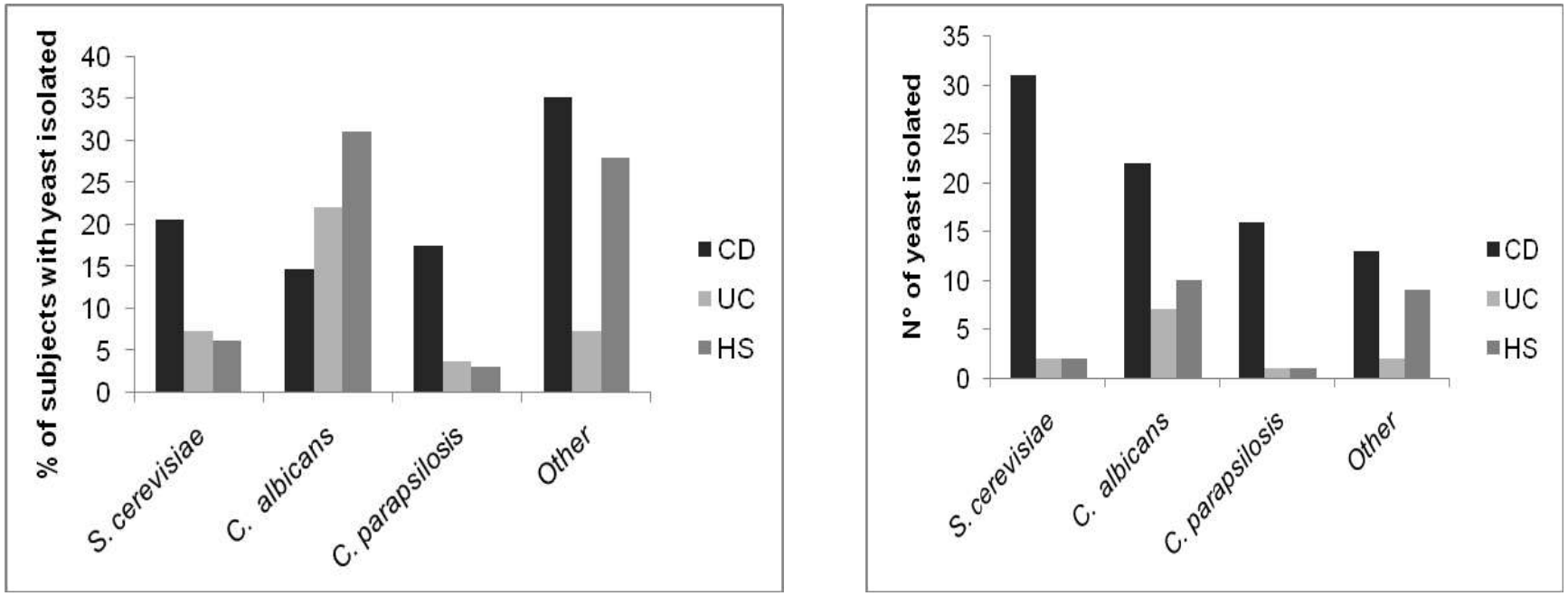
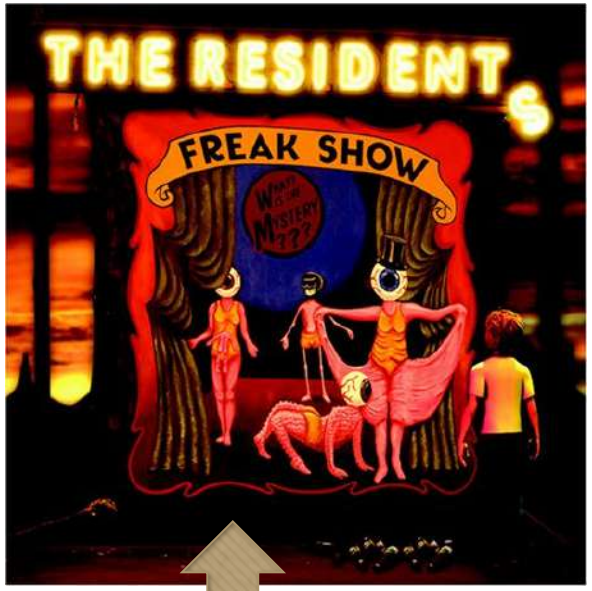


Fig.1. **Fungal flora composition in IBD patients and HS.** Distribution of yeast isolates (*n*=116) from faecal samples of the three groups studied (n=93 subjects); A) Number of yeast isolated per prevalent species in the three groups; B) Percentage of IBD and HS subjects with at least one isolate per prevalent yeast species..

De Filippo, C., Di Paola, M., Stefanini, I., Rizzetto, L., Berna, L., Ramazzotti, M., Dapporto, L., Rivero, D., Gut, I.G., Gut, M., Bayés, M., Legras, J.L., Viola, R., Massi-Benedetti, C., De Luca, A., Romani, L., Lionetti, P., Cavalieri, D. (2014). Population genomics of *Saccharomyces cerevisiae* human isolates: passengers, colonizers, invaders**.** ***BioX Riv***, 2014 doi:101101/001891,2013 – Web Preprint.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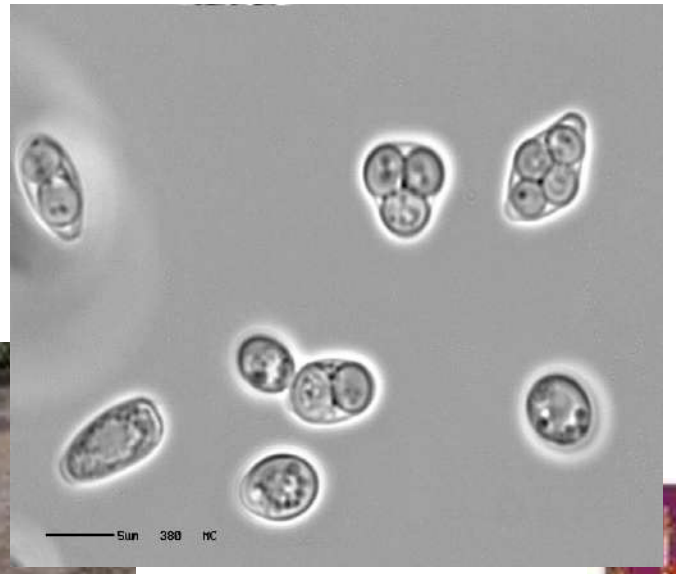
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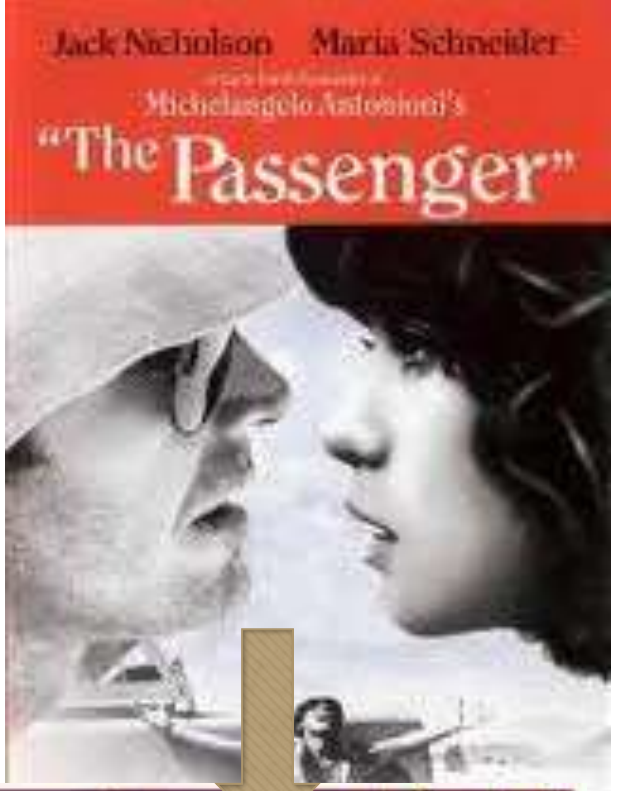
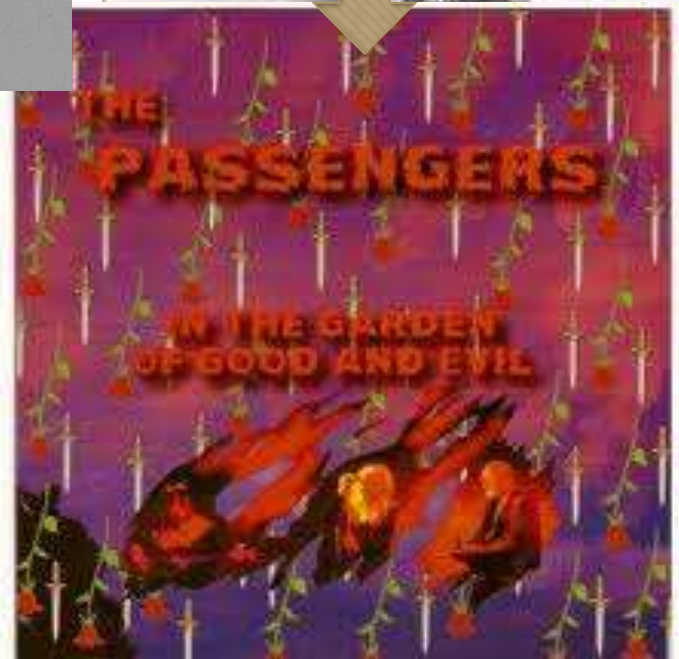

Jack Nicholson Maria Schneider
Michelangelo Antonioni's
"The Passenger"


WATER REPAIR
COLONIZE


THE PASSENGERS

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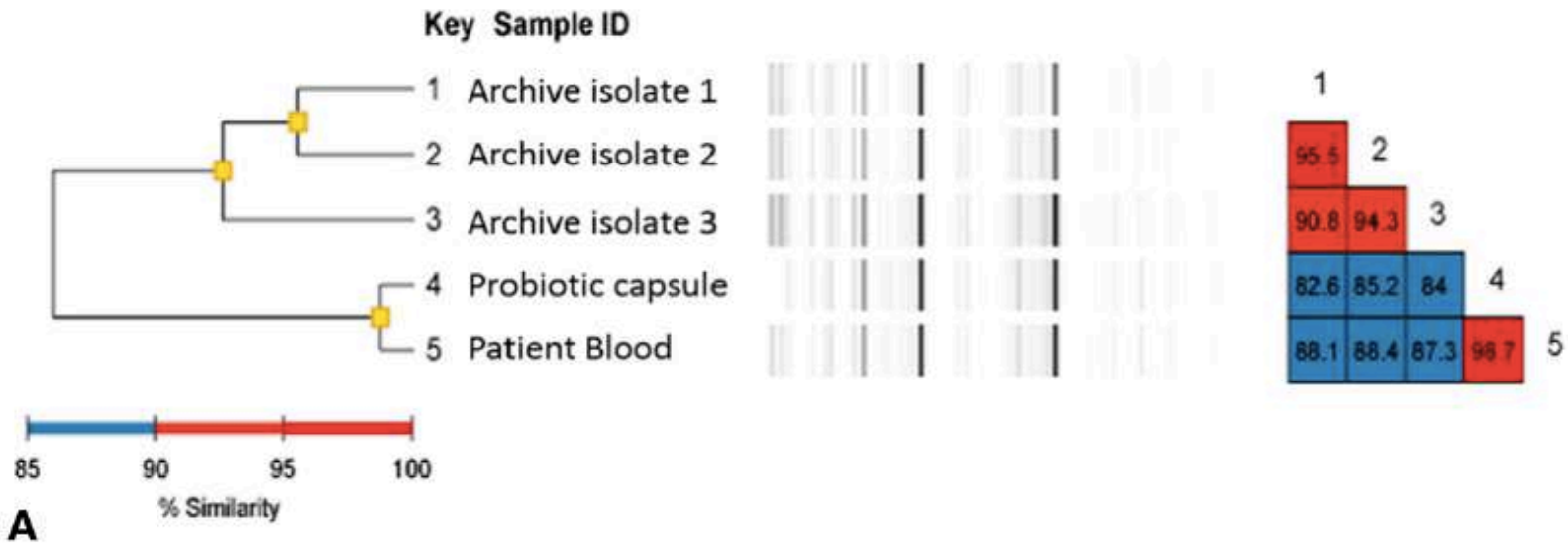
Saccharomyces boulardii probiotic-associated fungemia: questioning the safety of this preventive probiotic's use

Isabella W. Martin [a,1], Rita Tonner [b,1], Julie Trivedi [b], Heather Miller [a], Richard Lee [c], Xinglun Liang [d], Leo Rotello [e], Elena Isenbergh [e], Jennifer Anderson [e], Trish Perl [f,*], Sean X. Zhang [a,c,**]



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ORIGINAL ARTICLE

WILEY mycoses

Seven cases of *Saccharomyces* fungaemia related to use of probiotics

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TABLE 1 Salient clinical features of the patients with fungaemia due to *Saccharomyces cerevisiae*

Sr. No.	Demography	Underlying disease	Probiotic	Risk factors	Blood culture	Treatment	Outcome
1.	Neonate/M Chennai	Preterm (27 week) Birth weight (825 gm)	*S. boulardii* (as a hospital protocol for preterm neonates, twice daily via nasogastric tubes)	Umbilical CVC. Elemental diet, Total parenteral nutrition, Piperacillin - Tazobactam, fluconazole	*S. cerevisiae* (NCCPF 920006)	ABDC (0.7 mg/kg/day) Micafungin (2 mg/kg/day) CVC re-sited	Fungus cleared by 72 h, but died due to cardiac cause
2.	Neonate/M Chennai	Preterm (31 week) Birth weight (1.5 kg)	*S. boulardii* (as a hospital protocol for premature neonates, twice daily via nasogastric tubes)	Neonatal sepsis (day 14), elemental diet, meropenem, vancomycin	*S. cerevisiae* (NCCPF 920007)	Micafungin (2 mg/kg/day) × 14 day	Recovered
3.	75/M Kolkata	Respiratory failure, multiple episodes of bacterial sepsis and one episode of *Candida. tropicalis* candidaemia	*S. boulardii* (to prevent Antibiotic associated diarrhoea)	Intubation, meropenem, colistin, teicoplanin, caspofungin	*S. cerevisiae* (NCCPF 920004)	Caspofungin for 2 days. Discharged with oral voriconazole	Discharged
4.	37/M Kolkata	Polytrauma (sub-dural and sub-arachnoid haemorrhage withGCS-4), Diabetic, multiple episodes of infection with drug resistant bacteria, diarrhoea.	*S. boulardii* (due to diarrhoea)	Intubation, colistin, meropenem, vancomycin, metronidazole, rifabutin (after chest infection)	*S. cerevisiae* (NCCPF 920009)	Voriconazole for 2 days, then, caspofungin × 2 week	Recovered
5.	25/F Kolkata	32-week pregnancy, acute pancreatitis, acute kidney injury, LUCS performed	*S. boulardii* (to prevent antibiotic associated diarrhoea)	Haemodialysis, TPN, ceftriaxone for 2 days, replaced with meropenam, metronidazole, fluconazole for *Candida*	*S. cerevisiae* (NCCPF 920012)	Micafungin (100 mg) for 2 days, patient afebrile within 3 days, discharged with fluconazole 400 mg bd	Recovered
6.	66/F Kolkata	Cerebral stroke, prolonged hospitalization, multiple episodes of sepsis	*S. boulardii* (due to diarrhoea)	Prolonged hospitalization multiple antibiotics	*S. cerevisiae* (NCCPF 920005)	Treatment history not known	Not known
7.	32/M Kolkata	Superior vena cava syndrome due to mediastinal mass, fever, respiratory distress	No history of probiotics, referred from another hospital after 12 h' stay	Multiple antibiotics	*S. cerevisiae* (NCCPF 920010)	Caspofungin	Recovered

CVC, central venous catheterization; ABDC, amphotericin B deoxycholate; MDR, multidrug resistant; GCS, Glasgow coma scale; UTI, urinary tract infections; LUCS, lower uterine segment caesarean section.

MICROBIOTA

A member of the gut mycobiota modulates host purine metabolism exacerbating colitis in mice

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The commensal microbiota has an important impact on host health, which is only beginning to be elucidated. Despite the presence of fungal, archaeal, and viral members, most studies have focused solely on the bacterial microbiota. Antibodies against the yeast *Saccharomyces cerevisiae* are found in some patients with Crohn's disease (CD), suggesting that the mycobiota may contribute to disease severity. We report that *S. cerevisiae* exacerbated intestinal disease in a mouse model of colitis and increased gut barrier permeability. Transcriptome analysis of colon tissue from germ-free mice inoculated with *S. cerevisiae* or another fungus, *Rhodotorula aurantiaca*, revealed that *S. cerevisiae* colonization affected the intestinal barrier and host metabolism. A fecal metabolomics screen of germ-free animals demonstrated that *S. cerevisiae* colonization enhanced host purine metabolism, leading to an increase in uric acid production. Treatment with uric acid alone worsened disease and increased gut permeability. Allopurinol, a clinical drug used to reduce uric acid, ameliorated colitis induced by *S. cerevisiae* in mice. In addition, we found a positive correlation between elevated uric acid and anti-yeast antibodies in human sera. Thus, yeast in the gut may be able to potentiate metabolite production that negatively affects the course of inflammatory bowel disease.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal (GI) tract that includes Crohn's disease (CD) and ulcerative colitis (UC). Unrestricted inflammation at intestinal sites leads to malabsorption of nutrients, severe abdominal pain, and increased chance of developing colorectal cancers (*1*). Although current therapies such as immunosuppression, intestinal resections, and antibiotics ameliorate symptoms, there is no cure for this disease. Emerging evidence supports a role for the microbiota in the modulation of IBD. Multiple studies have demonstrated that both community membership and abundance within the microbiota in patients with IBD are different from those of healthy individuals (*2*, *3*). Moreover, probiotic treatment during experimental colitis can alleviate disease (*4*–*6*). The human microbiota is a complex ecosystem composed of bacteria, fungi, archaea, and viruses; however, most studies to date have focused solely on bacterial members and their involvement in IBD (*2*, *3*).

Fungi comprise a diverse kingdom of eukaryotic organisms composed of yeasts, molds, and mushrooms. Several yeast species have been identified in the GI tract, where they are estimated to make up 0.1% of the intestinal microbiota (*7*). Common members include *Candida*, *Saccharomyces*, *Aspergillus*, *Cryptococcus*, and *Rhodotorula*, which indicate the existence of a diverse resident fungal community (*8*–*14*). Fungi are large, complex organisms known to act opportunistically during immune-mediated and antibiotic therapy (*15*–*17*). There are several clinical and experimental indications that yeast might influence intestinal inflammation. The first was the discovery of elevated anti–*Saccharomyces cerevisiae* antibodies (ASCAs) in the serum of CD patients (*16*, *18*), which suggests that an aberrant immune response to yeast might be involved in IBD progression. Patients suffering from IBD are often prescribed antibiotics to kill bacteria thought to drive the chronic inflammatory response (*19*–*22*). Because yeasts are not targets of commonly used antibiotics, a well-documented side effect of extended antibiotic use is the overgrowth of fungal species (*23*–*25*). Moreover, yeasts are a common component of many foods, which might provide daily exposure to these organisms. Polymorphisms in genes such as *CARD9* and *CLEC7A* (dectin-1) that function in fungal recognition by the host have been described in patients who suffer from increased fungal infections (*26*–*28*). Deletion of these genes in mice leads to worsened intestinal disease (*29*, *30*). Thus, there is evidence in both mouse models and humans that supports a role for fungi in intestinal disease, yet the mechanisms by which this occurs remain poorly defined. Here, we investigate a role for *S. cerevisiae* in the pathogenesis of colitis.

The study of resident fungal communities is still in its infancy; however, there are a handful of papers that have surveyed fungal populations in patients with IBD (*8*, *12*, *31*). Several of these studies have reported a loss of bacterial diversity with a concomitant increase in fungal variety and load during IBD (*8*, *32*). Similarly, the most recent and largest patient survey of fungal communities identified a higher fungal-to-bacterial ratio in patients with IBD (*12*), further supporting the hypothesis that increases in fungal load might be associated with disease. Several species of fungi have now been identified from both colitogenic and healthy individuals, including *S. cerevisiae*, *Candida albicans*, *Penicillium italicum*, *Rhodotorula aurantiaca*, and *Malassezia sympodialis* (*8*, *12*, *14*). These species belong to two fungal phyla, the Ascomycota and Basidomycota, which dominate the intestinal fungal community. To investigate how different yeast species identified in the human GI tract influence intestinal disease, we chose one organism from each of these fungal phyla that are readily available, easily cultured, and present in individuals with IBD—*S. cerevisiae*, a member of the Ascomycota, and *R. aurantiaca*, a member of the Basidomycota. *S. cerevisiae* represents one of the most highly abundant and most commonly detected fungal members in the human GI tract and is also found in food and the environment (*12*, *14*), making it highly relevant to human biology. Moreover, we studied a wild, prototrophic, critical component to prevent intestinal disease in mouse models and humans (*38*, *39*). Thus, our data suggest that a common member of the intestinal and environmental mycobiota, *S. cerevisiae*, can worsen intestinal disease by enhancing gut epithelial leakage.

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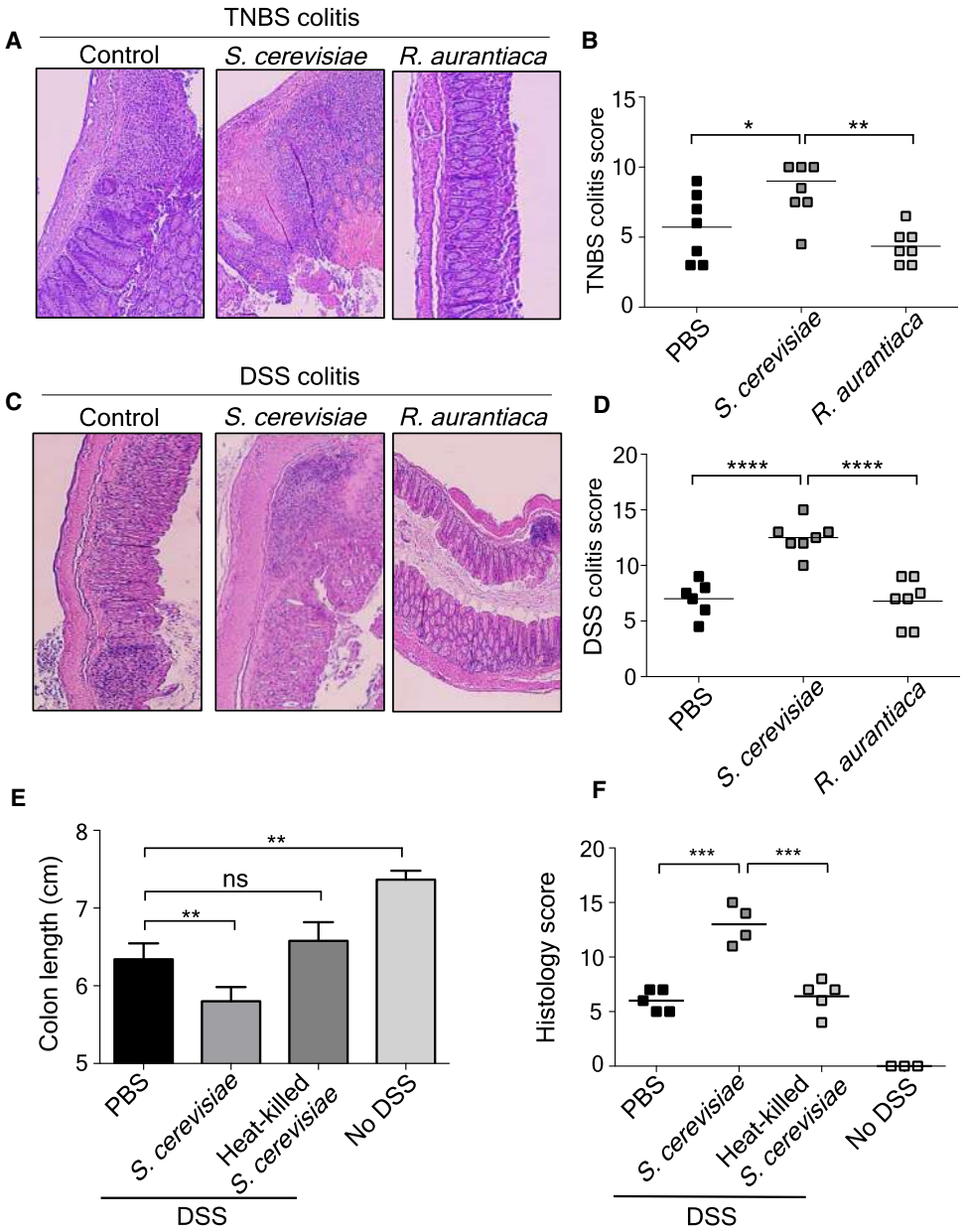

Fig. 1. *S. cerevisiae*, but not *R. aurantiaca*, exacerbates intestinal disease in two mouse models of colitis. (**A** and **B**) C57Bl/6 specific pathogen–free (SPF) mice were orally gavaged daily for 7 days with 10^6 of *S. cerevisiae* (*n* =

S. cerevisiae enhances degradation of purine produced by intestinal epithelia

Other major gene expression pathways influenced by yeast colonization in germ-free mice were host metabolic pathways (fig. S2, B and C). The microbiota contributes important functions to their mammalian hosts, including colonization resistance and induction of immune responses; however, one of the most well-known roles for the microbiota is the breakdown of dietary components for use by host metabolism (*40*). How commensal fungi influence metabolism is largely unknown. Therefore, we generated a fecal metabolomic profile using gas chromatography–mass spectrometry (GC-MS) from SPF, germ-free, and *S. cerevisiae* or *R. aurantiaca* monoassociated animals to determine how yeast colonization might influence metabolism. SPF animals are markedly different from the other three groups, such that yeast monoassociated animals cluster more closely with germ-free animals (Fig. 3A). Despite the similarity between the metabolomic profile of yeast monocolonized and germ-free animals, there were 20 metabolites that were influenced by yeast colonization (Fig. 3B). Many of the metabolites found to be elevated in germ-free mice were sugars such as ribitol and mannitol (fig. S3, A and B). These sugars were depleted upon monoassociation with either yeast species and likely indicated the use of these sugars by colonizing yeast.

Of the few metabolites that differed between *S. cerevisiae* and *R. aurantiaca*, five were part of the purine degradation pathway (Fig. 3C and fig. S3C) and included adenosine, adenine, xanthine, hypoxanthine, and uric acid. Uric acid is the oxidative product of hypoxanthine and xanthine through the action of the enzyme xanthine oxidase (XO) (*41*). Most yeast species lack XO; rather, they convert hypoxanthine to xanthine via xanthine dioxygenase. However, *S. cerevisiae* lacks all of the enzymes necessary to catabolize purines (*42*). These observations support the