

“Fungal Microbiomes in Health and Disease”

The 3rd Microbiome R&D and Business Collaboration Forum: USA
San Diego, CA Sept. 2015

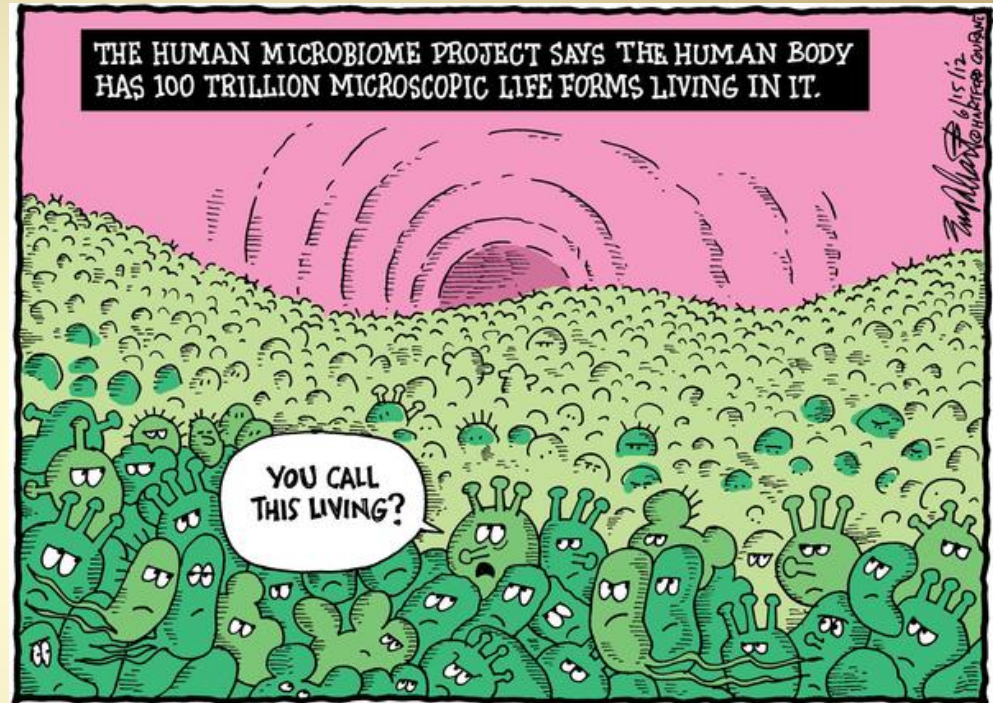
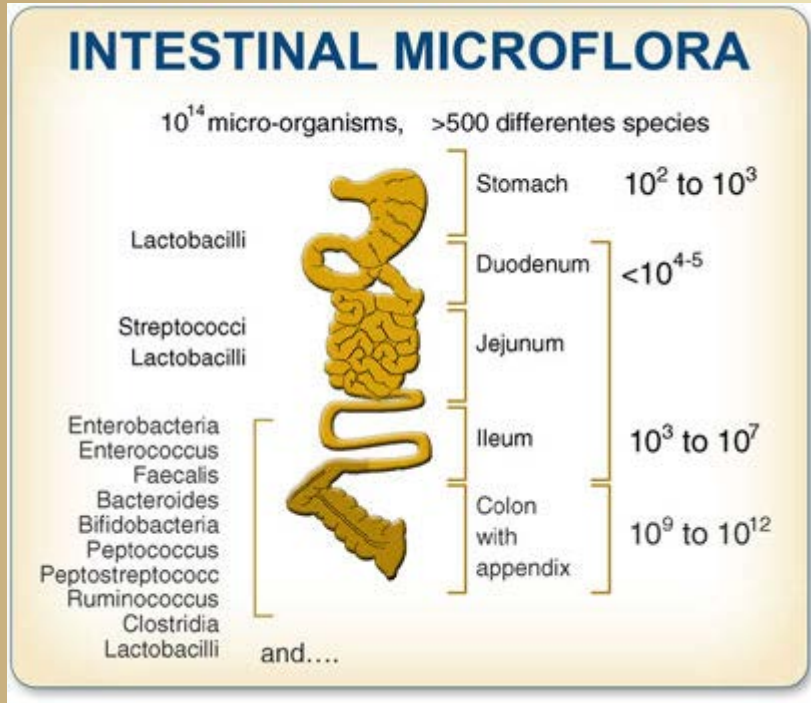
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The Intestinal Microbiome



... but it's all about bacteria (and viruses, as we've heard...)

The term “microbiome” has become nearly synonymous with “bacterial microbiota”
People have known for decades that fungi are part of the normal commensal microbiota, but little has been done to investigate this
Many reasons to believe that fungi might be particularly relevant in this environment

Fungal abundance

It has been estimated that as much as 25% of the world's biomass is fungi



Ophiocordyceps camponoti-balzani, grows out of a "zombie" ant's head in a Brazilian rain forest



Mycena fera A luminescent fungus in the South American rainforests



Ryan von Linden/ New York Department of Environmental Conservation

Geomyces destructans "White-nose syndrome" threatening to wipe out bats in North America



Hymenoscyphus pseudoalbidus (Ash dieback disease) virulent fungal pathogen of ash trees killing off ash trees across Europe



Batrachochytrium dendrobatidis, killing off the world's frogs.



Active growth of fungi in soil around hot springs (>65°C)



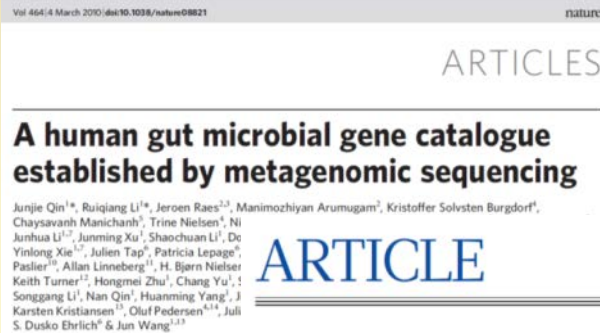
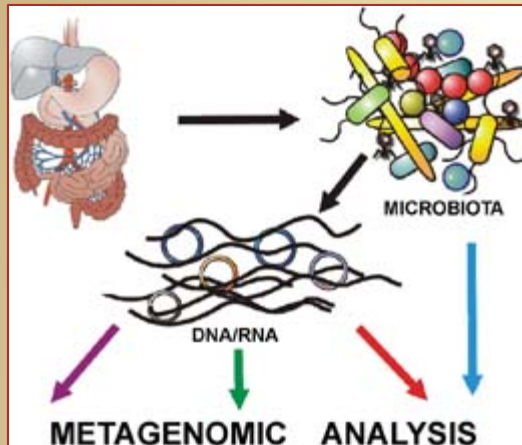
Active growth of fungi in the arctic winter (below 2°C).



Live fungi in the upper atmosphere (>30,000 ft).

So, what about fungi in the gut?

Recent intestinal “metagenome” studies



Enterotypes of the human gut microbiome

Manimozhayan Arumugam^{1*}, Jeroen Raes^{1,2*}, Eric Pelletier^{3,4,5}, Denis Le Paslier^{1,4,5}, Takuji Yamada¹, Daniel R. Mende¹, Gabriel R. Fernandes^{1,6}, Julien Tap^{1,7}, Thomas Bruls^{3,4,5}, Jean-Michel Batto⁷, Marcelo Bertalan⁸, Natalia Borruel⁹, Francesc Casellas⁹, Leyden Fernandez¹⁰, Laurent Gautier⁸, Torben Hansen^{11,12}, Masahira Hattori¹³, Tetsuya Hayashi¹⁴, Michiel Kleerebezem¹⁵, Ken Kurokawa¹⁶, Marion Leclerc⁷, Florence Levenez⁷, Chaysavanh Manichanh⁹, H. Bjørn Nielsen⁸, Trine Nielsen¹¹, Nicolas Pons⁷, Julie Poulain³, Junjie Qin^{1,7}, Thomas Sicheritz-Ponten^{8,18}, Sebastian Tims¹⁵, David Torrents^{10,19}, Edgardo Ugarte³, Erwin G. Zoetendal¹⁵, Jun Wang^{1,7,20}, Francisco Guarner⁹, Oluf Pedersen^{11,21,22,23}, Willem M. de Vos^{15,24}, Soren Brunak⁶, Joel Doré², MetaHIT Consortium†, Jean Weissenbach^{3,4,5}, S. Dusko Ehrlich⁷ & Peer Bork^{1,25}

Human Microbiome Project data

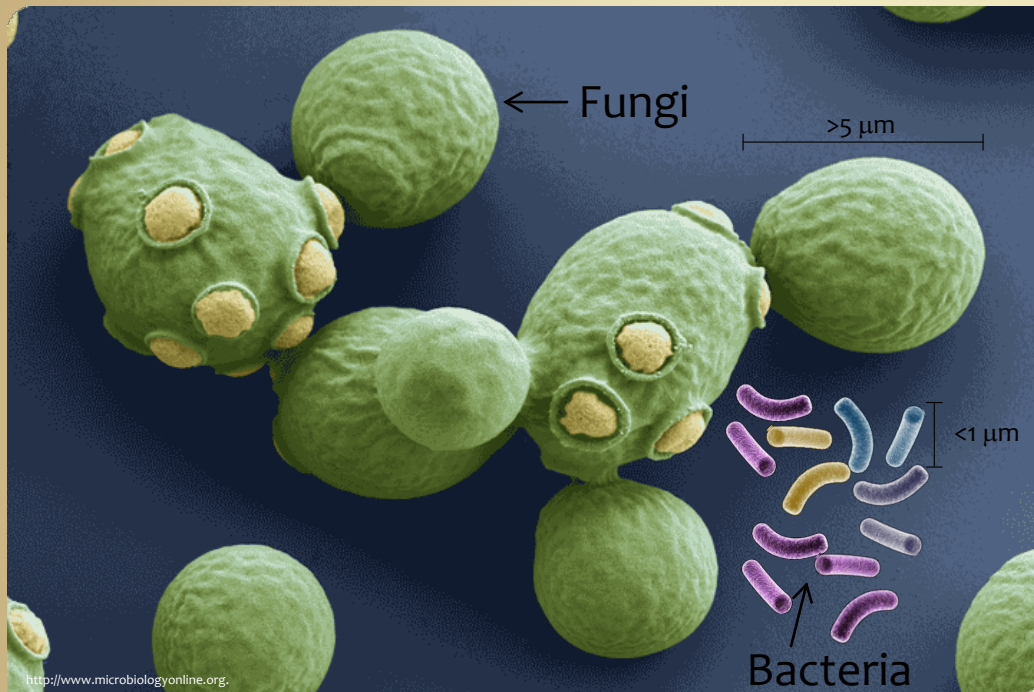
Conclude that:

99.1% of the genes are of bacterial origin (using a 90% match criteria)

Only 0.1% of eukaryotic origins.

Doesn't suggest that fungi are a particularly significant fraction of intestinal microbial population. But....

Reasons to think the mycobiome has been underestimated

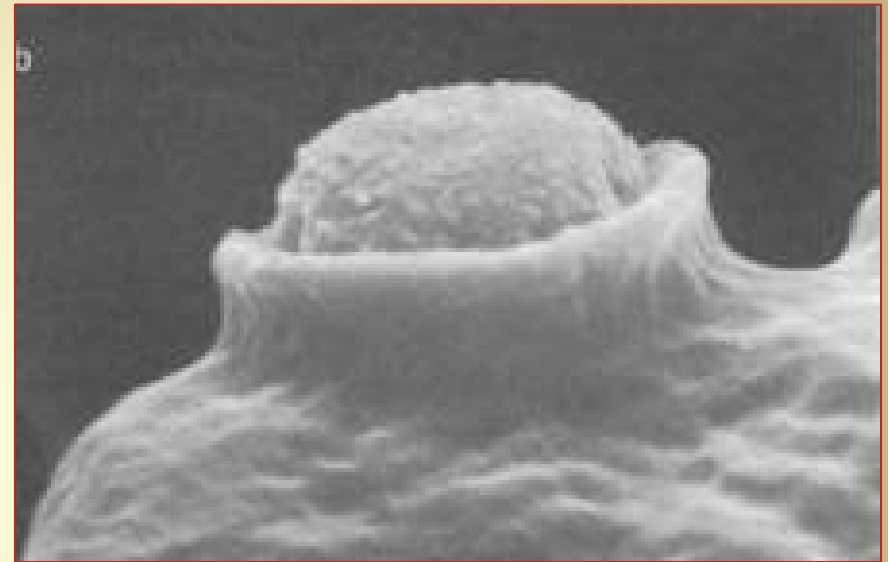
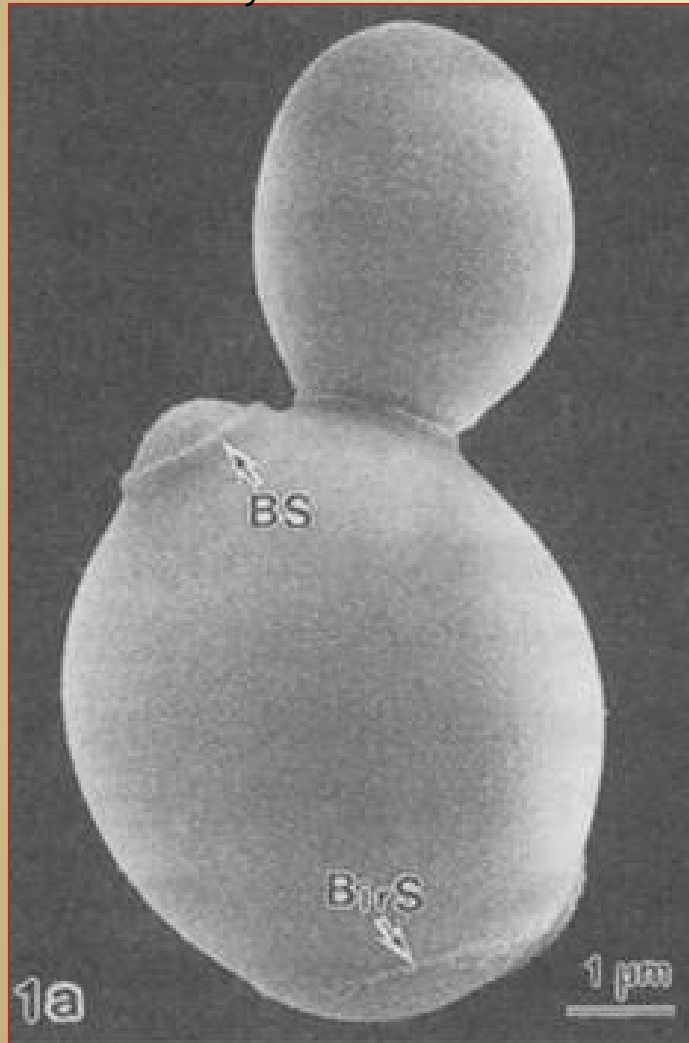


Although there are many more bacteria than fungi in the gut, we suspect that the contribution of commensal fungal communities to health and disease are understudied.

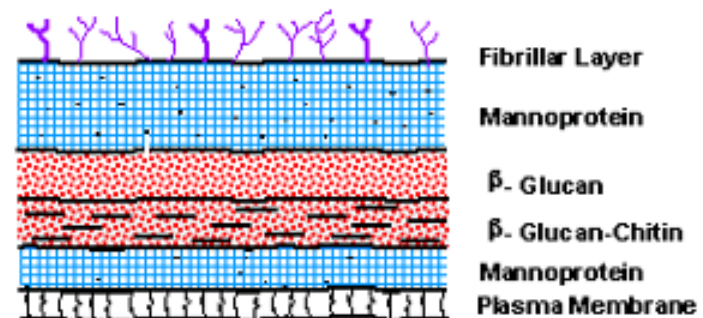
- The average fungal cell is >50X larger than an average bacterium.
- Fungal DNA is encased in a cell wall that is harder to extract DNA from than bacterial cell walls.
- Fungal sequences are rarer in public databases than bacterial sequences.
- Even “rare” bacteria are now being demonstrated to have significant effects on mammalian physiology & immunology

Fungal Cell Wall Structure

Saccharomyces cerevisiae



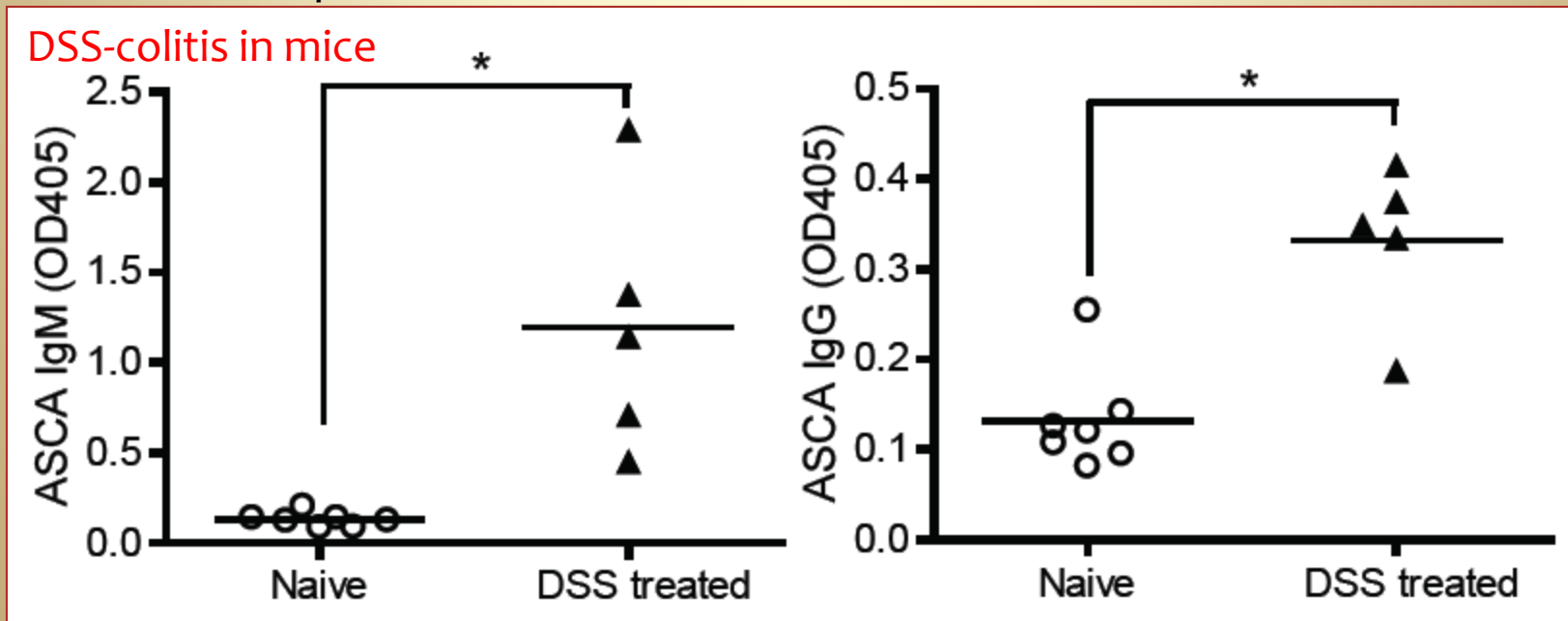
Structure of Yeast Cell Wall



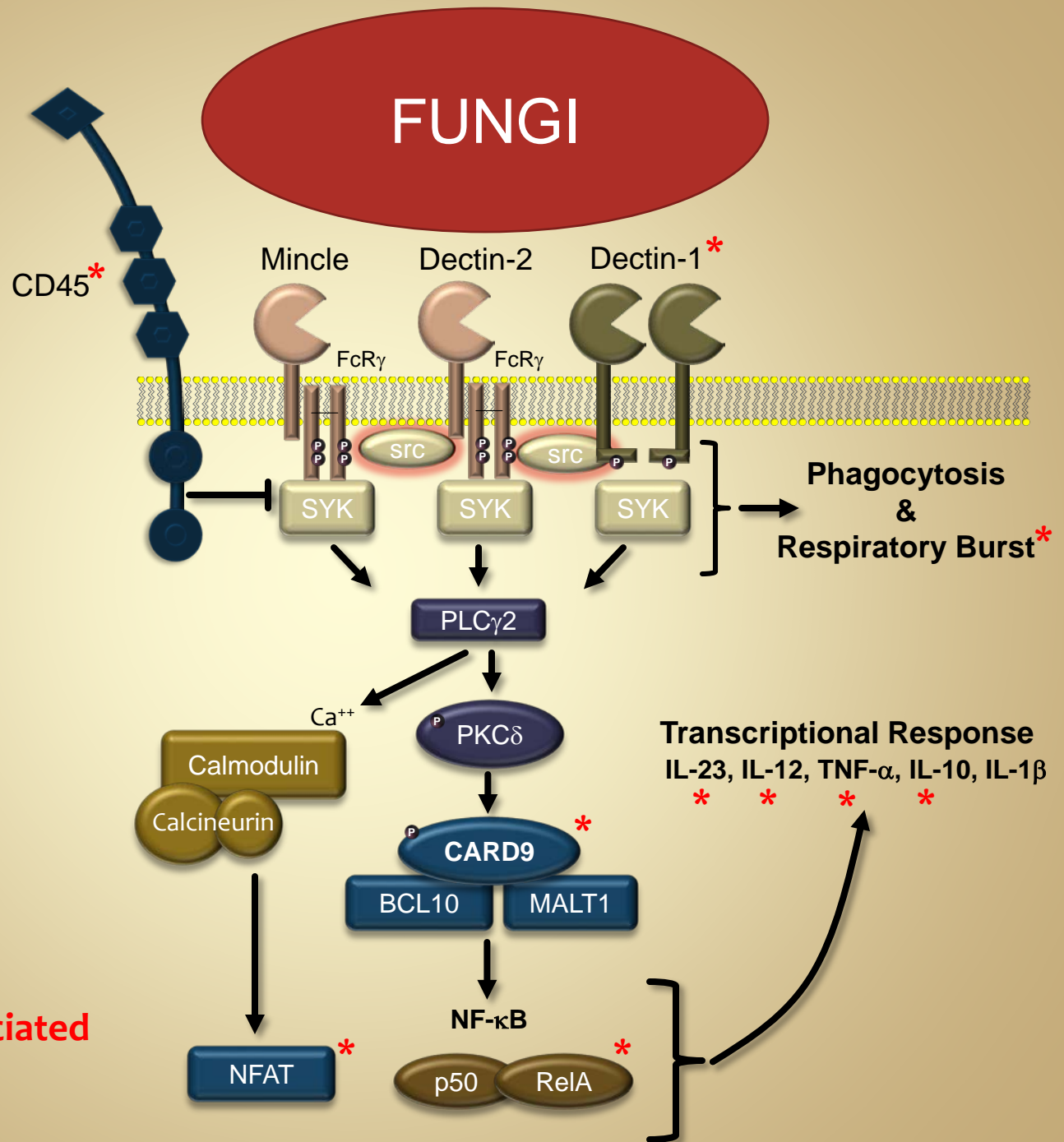
Mannan
 β -glucan
Chitin

“ASCA” Anti-Saccharomyces Cerevisiae antibodies

- The first biomarker that was capable of identifying the majority of patients with Crohn’s disease.
- 50-60% of patients with Crohn’s disease have circulating antibodies to fungi (ASCA).
- The antigen is yeast mannan and is part of the cell wall of nearly all fungi.
- More recently, anti-glucan and anti-chitin antibodies have also been measured in serum from IBD patients.



Innate Immune Recognition of Fungi

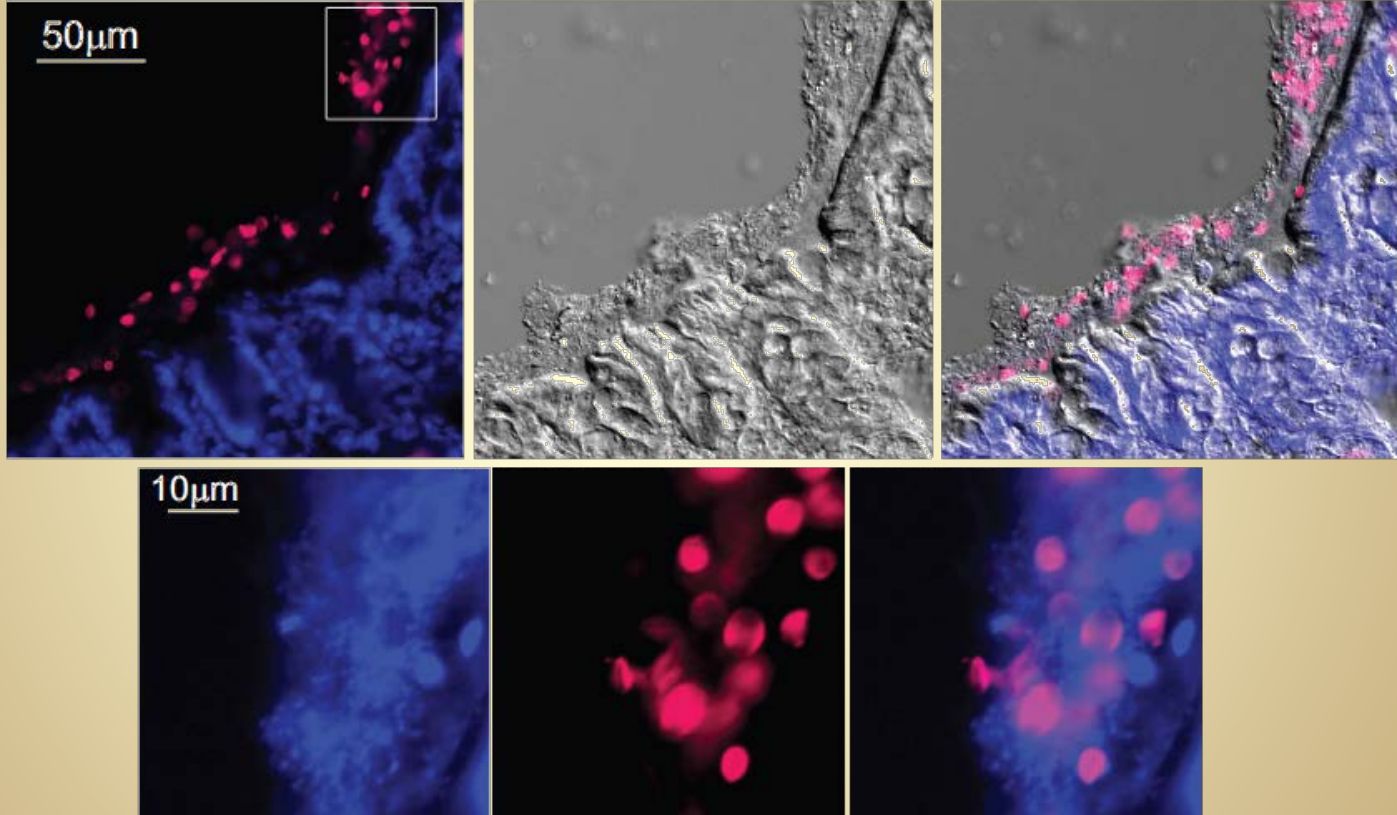


* Polymorphisms associated with IBD

Recognition of Intestinal Fungi by Dectin-1



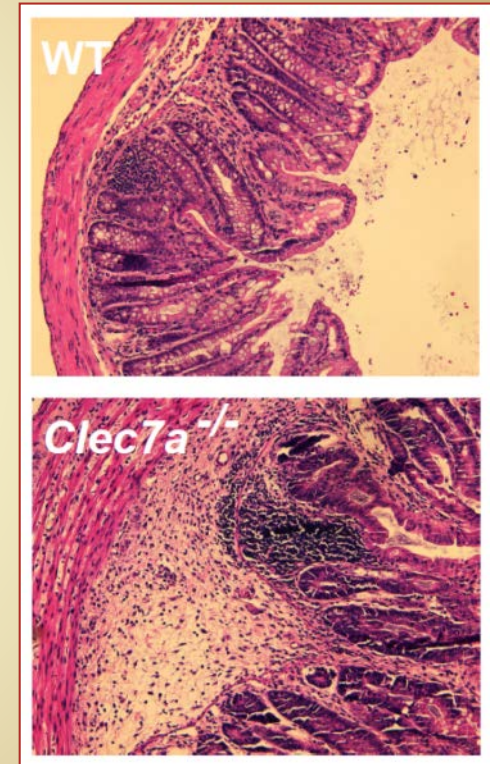
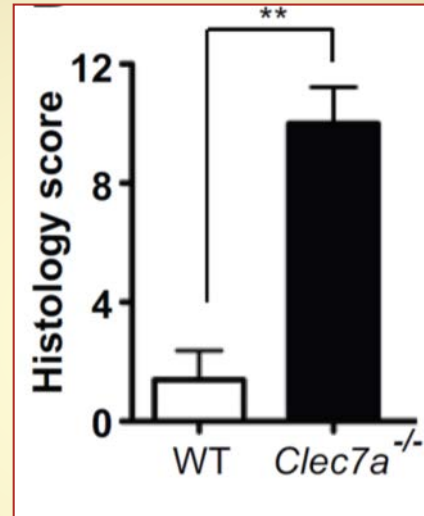
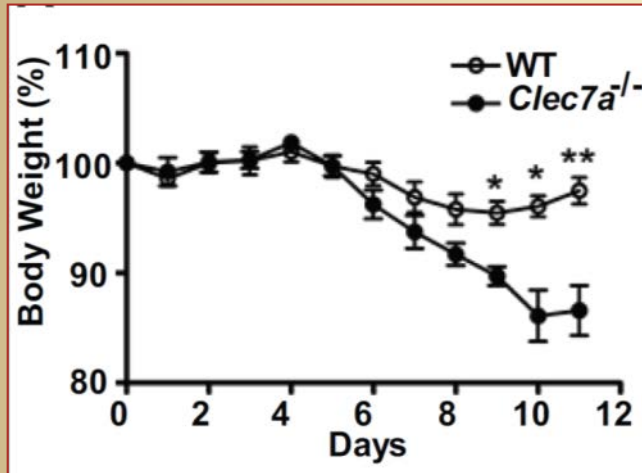
Iliyan Iliev, Ph.D.



Recombinant,
Fluorescently
Labeled
Soluble
Dectin-1

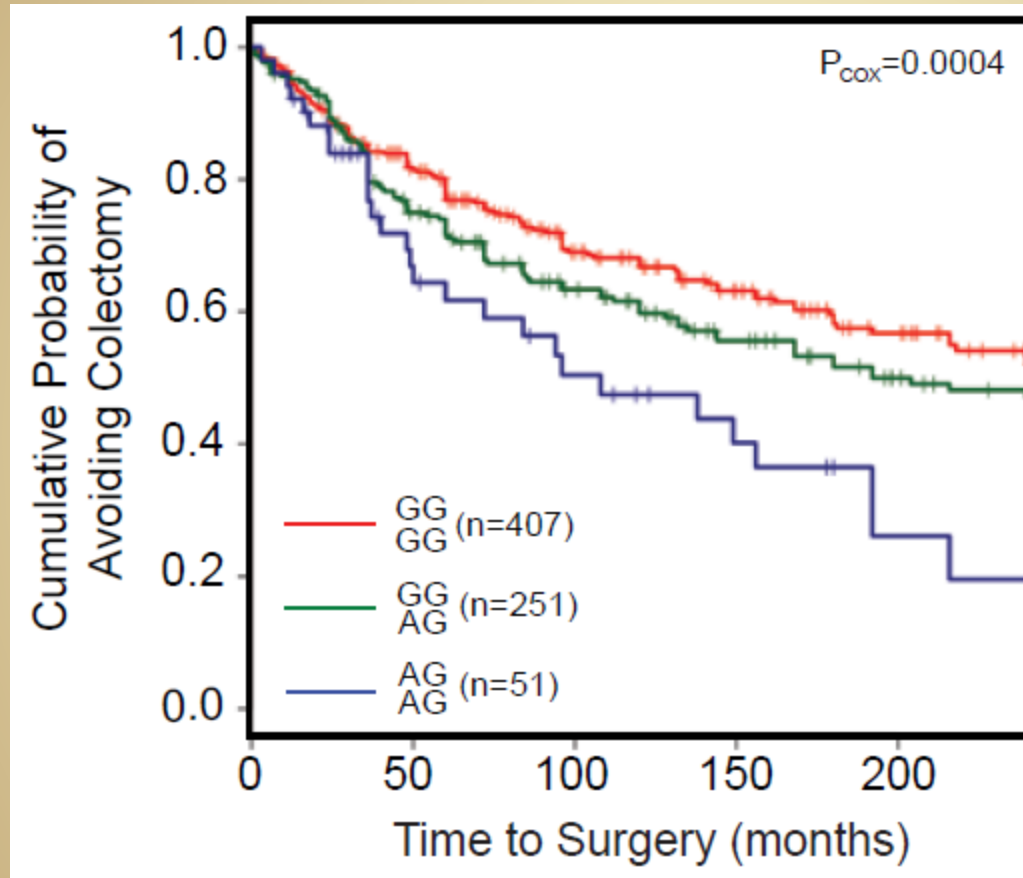
RED = Soluble Dectin-1 Probe
Blue = DAPI

Dectin-1 knockout mice are highly susceptible to DSS colitis



Due to failure to control mucosal invasion of intestinal wall by opportunistic pathogenic fungi when the barrier is damaged by DSS

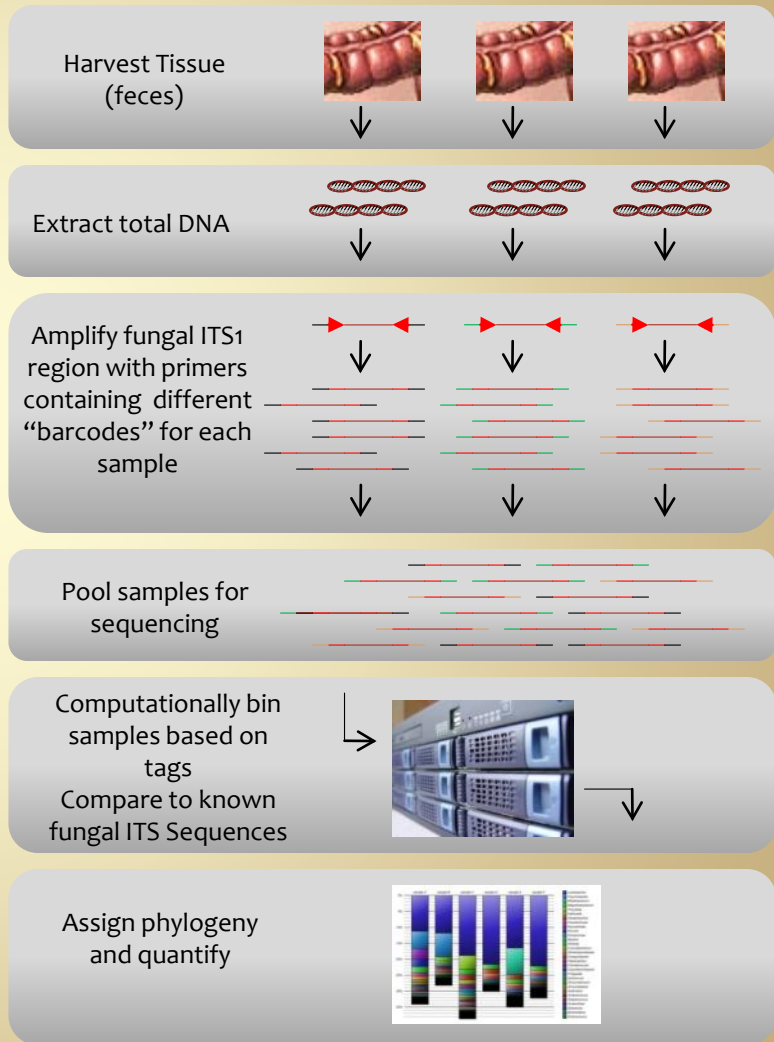
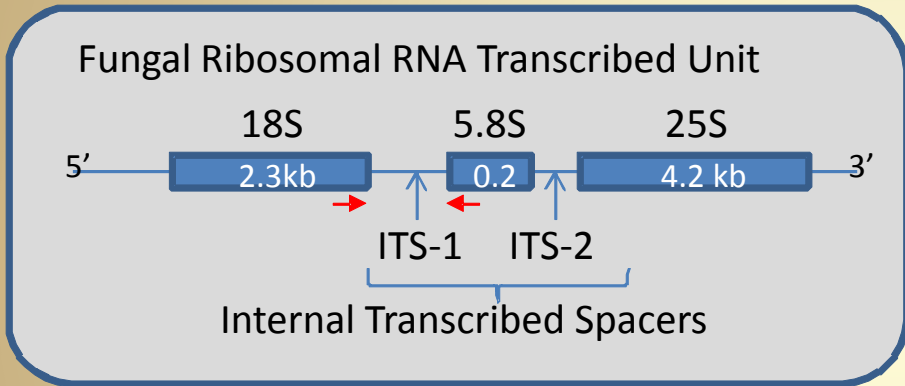
Polymorphisms in the gene for Dectin-1 are associated with severity of disease in Ulcerative Colitis patients



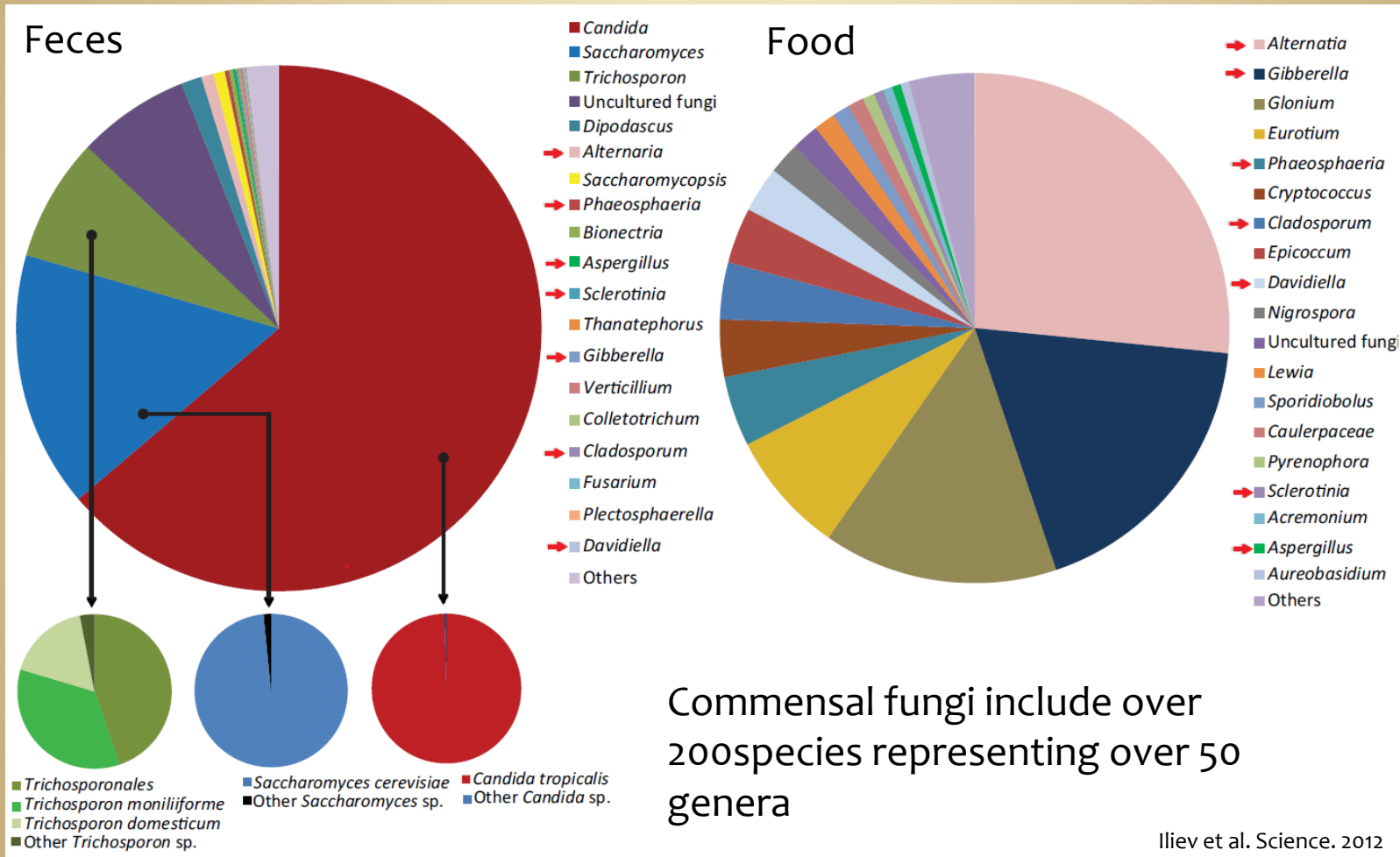
← Over 20 year, folks with this Dectin-1 variant are far more likely to require surgery

What Fungi are in a mouse gut?

Deep Sequencing of murine fecal Fungal rDNA



What Fungi are in a mouse gut?



Challenges to scaling this up to embark on human studies...

Database Challenges

- There are approximately 3.1 million “fungal” sequences in GenBank. Around 900K are annotated as including an “Internal Transcribed Spacer”.
 - More than 300K of these are annotated as “uncultured genus” X.
 - More than 75K are simply annotated as “uncultured fungus”.
- It has been estimated that as many as 10-20% of the GenBank fungal entries are misidentified.
- To date, there is no definitive fungal sequence/nomenclature reference database.
- Although there are many disparate efforts underway to produce an acceptable “gold standard” database, this is undoubtedly many years (decades?) away.

Aspergillus/Eurotium

By sequencing, these are the same organism. But historically, they've been called different things because they look different.

Eurotium herbariorum



Aspergillus glaucus



ITS1 in Genbank

12 times entered as *Eurotium*

4 times entered as *Aspergillus*

<i>Eurotium herbariorum</i>		<i>Aspergillus glaucus</i>
GU319986.1	JN942870.1	KC485448.1
JF922029.1	AY373887.1	JQ724459.1
JN985120.1	EF652053.1	JQ724404.1
JN938995.1	EF652052.1	JQ724365.1
JN938918.1	EF652051.1	
AB008402.1	EF652047.1	

Sexual – teleomorph

Asexual – anamorph

Kingdom: Fungi
Phylum: Ascomycota
Class: Eurotiomycetes
Order: Eurotiales
Family: Trichocomaceae

At least these share this much in common.

This is not always the case

Our Approach to Building a “Custom Database”

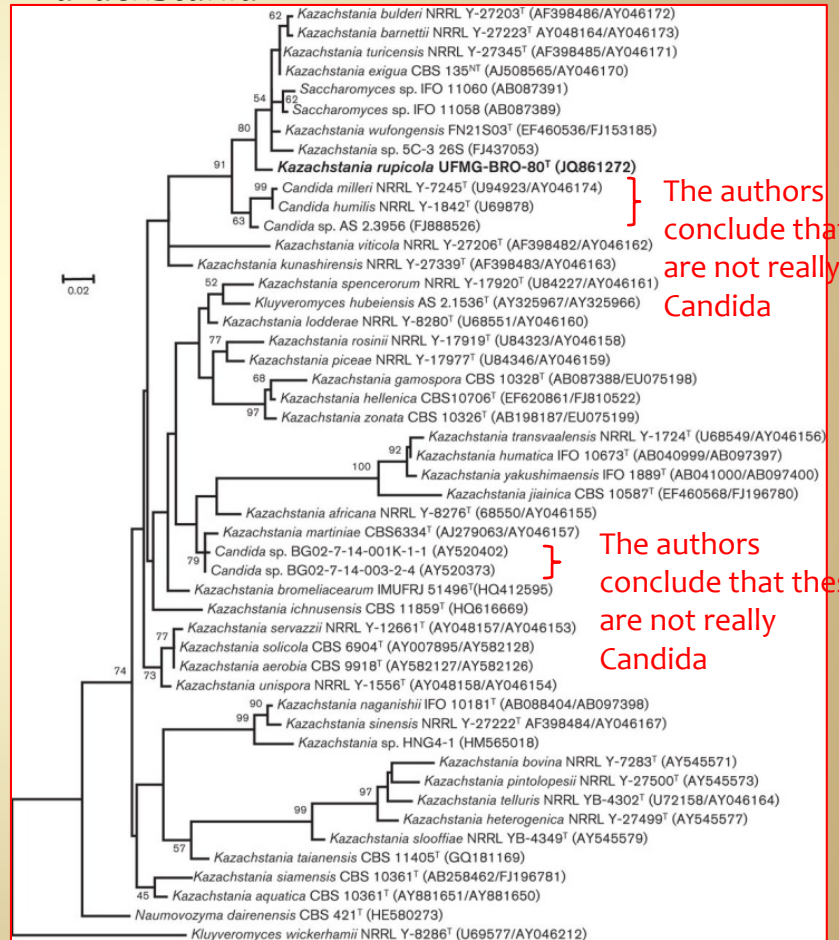
1 – Use existing databases to get a list of genus names that we should be interested in.

2 – Manually go to the literature to find curated phylogenetic trees comprehensively covering the ITS1 variation of the genus

3 – Build database from these sequences and do not include alternate names/sexual forms.

We end up with a small database (≈2000 sequences) that is **computationally fast** and **more reliable** in naming the fungi.


Kazachstania




The authors conclude that these are not really *Candida*


The authors conclude that these are not really *Candida*


We've gone through several rounds of this...

Missed – not in the mouse data originally used to train & build the database.  Manually add this genus in next round.

“unclassified” that were correctly classified by our database 

Teleomorph name of *Alternaria* – Our database correctly grouped these 

Teleomorph name of *Fusarium* – Our database correctly grouped these 

Teleomorph name of *Cladosporium* – Our database correctly grouped these 

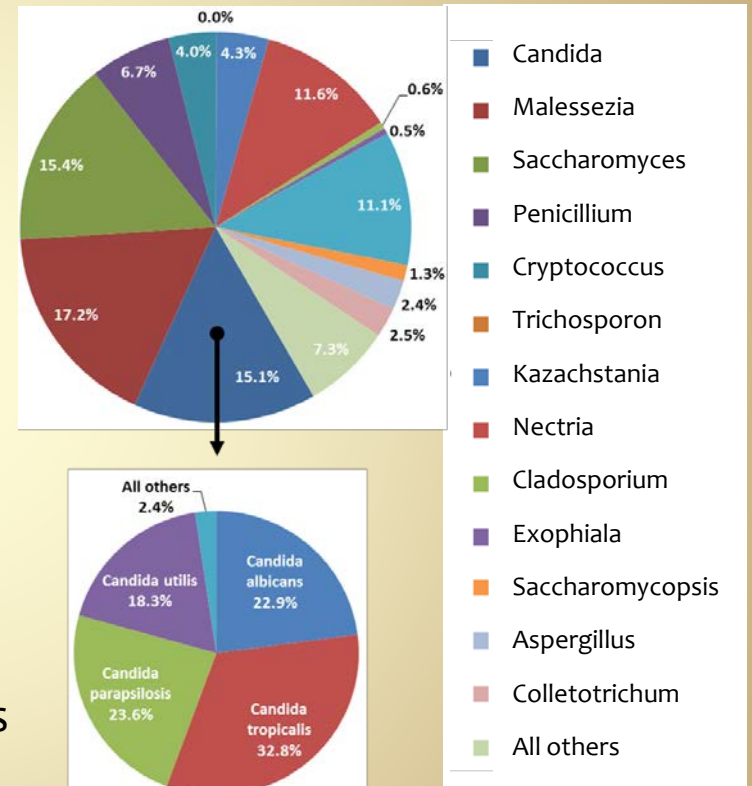
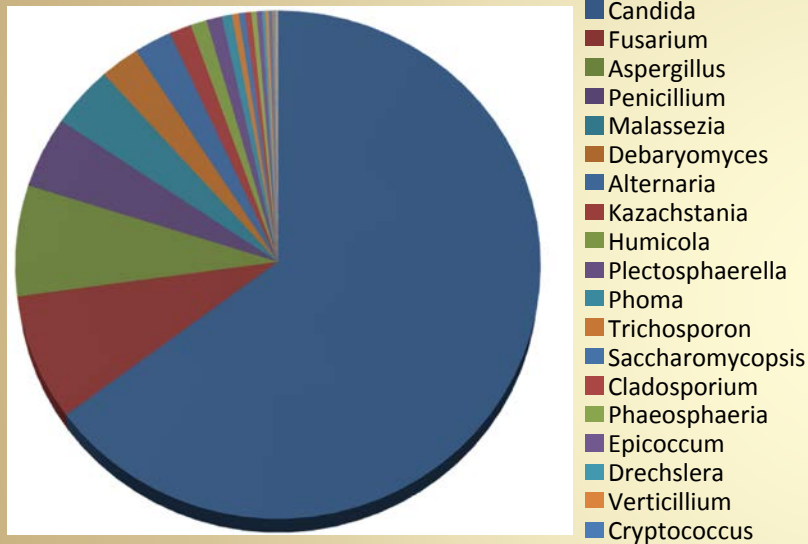
Taxon	NIH Database	In-house Curated
Malassezia	23.19%	24.80%
Fusarium	14.94%	15.98%
Candida	14.55%	17.54%
Saccharomyces	8.63%	8.29%
Cladosporium	7.54%	9.20%
Phoma	6.54%	8.41%
Alternaria	3.40%	4.23%
Epicoccum	2.79%	1.15%
Filobasidium	2.63%	-
Kazachstania	2.40%	5.06%
Nectria	2.19%	0.12%
unclassified_Capnodiales_genus	2.03%	-
Saccharomycopsis	1.92%	1.85%
unclassified_Hypocreales_genus	1.74%	-
unclassified_Saccharomycetaceae_genus	1.46%	-
unclassified_Saccharomycetales_genus	1.12%	-
Coniosporium	1.03%	0.99%
Cryptococcus	0.99%	1.92%
Lewia	0.36%	-
Exophiala	0.27%	0.25%
Aspergillus	0.15%	0.14%
Verticillium	0.05%	-
Gibberella	0.04%	-
Penicillium	0.02%	0.01%
Davidiella	0.01%	-
Phaeosphaeria	0.01%	0.01%

Current version available at: <https://risccweb.csmc.edu/microbiome/thf/>

Current assessment of intestinal diversity...

Human Feces (n=9 children, 2014)

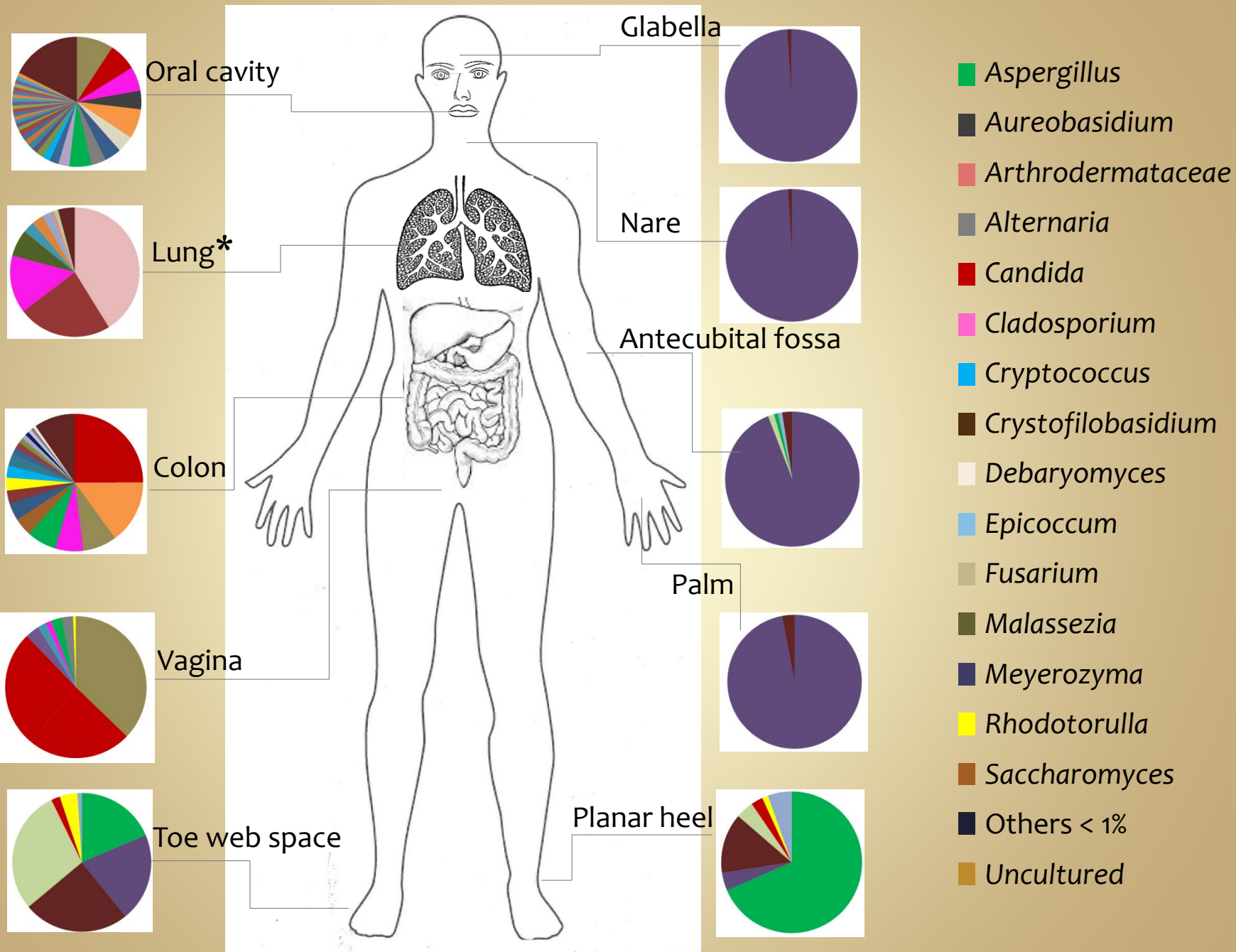
Mouse feces (n=21, 2014)



Typically 80-90% mapping efficiency for datasets derived from human or mouse GI tract.



Phil Frykman, MD, Ph.D.



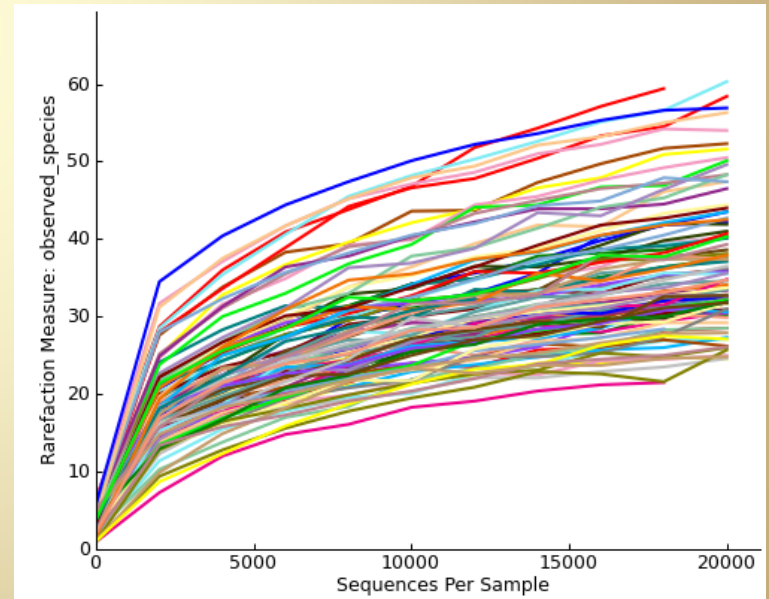
What about fungi directly associated with the mucosa in humans?

>100 samples from mucosal washes taken during colonoscopy at Cedars-Sinai

- Collected from both the Cecum and Sigmoid colon.

- Enriched for organisms associated with the wall of the gut

- Can be very different from fecal material



And finally, the people who did the work...

Underhill Lab & F. Widjaja Foundation Inflammatory Bowel & Immunobiology Research Institute



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