Evolutionary and Comparative Fungal CAZomics.

A-5

Annotation, comparative and evolutionary analysis of fungal Carbohydrate Active enZymes (CAZymes).

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FUNGI

The most abundant source of available whole genomes in Eukaryotes (about 40 genomes already available, > 50 more in the short term).

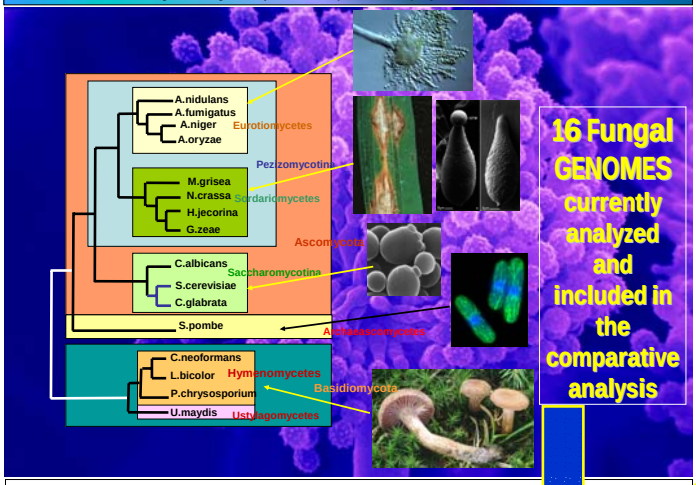
A huge Biodiversity (~ 75,000 species already described, upper estimate of 1.5 million species [1,2].

High variability and diversity of lifestyles (sexual / asexual…), lifeforms (uni- pluri cellular) and ecotypes (pathogens, parasites, saprophytes, symbionts…).

Available Fungal genomes already cover a diverse sample of the huge Biodiversity.

Fungi have close relationships with carbohydrates and are known to be excellent source of CAZymes in particular for industrial applications.

[1] Hawksworth DL: **The fungal dimension of biodiversity: magnitude, significance, and conservation.** *Mycological Research* 1991, **95**:641-655.
[2] Hawksworth DL: **The magnitude of fungal diversity: the 1.5 million species revisited.** *Mycological Research* 2001, **105**:1422-1432.



16 Fungal GENOMES currently analyzed and included in the comparative analysis

CAZymes (Carbohydrate Active enZymes)

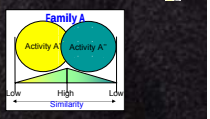
- Enzymes forming, modifying and degrading glycosidic bonds.
- Found in all kingdoms of life (including viruses)
- Particularly important for the biology of species having close relations with sugars (i.e. Fungi).
- Widely used in the industry (food, agriculture, pulp & paper, biomass conversion, etc)
- > 30 % of world enzyme's market.
- Involved in human and animal pathologies.
- Typically 2-3 % of a genome in Eukaryotes (with considerable variations).

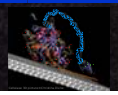
A given CAZyme family Can Feature :

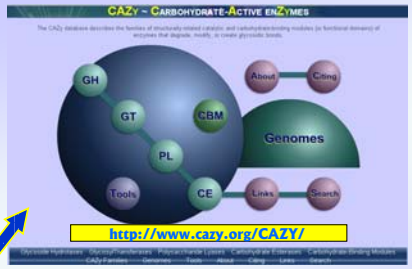
Variability of modular structure

Variability of subtract / product specificity

→ Classical structural and functional annotation based on best BLAST hits is not efficient in that case and is often misleading.







CAZy DB

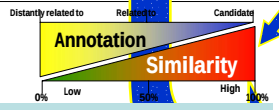
>50,000 curated entries corresponding to candidate CAZymes.
Classified into families :

- **106** Glycoside-Hydrolases (**GH**).
- **87** Glycosyl-Transferases (**GT**).
- **18** Polysaccharide-Lyases (**PL**).
- **14** Carbohydrate-Esterases (**CE**).
- **45** ancillary Carbohydrate Binding Modules (**CBM**).

CAZy Annotation

Structural Annotation takes into account the modular organization. Assignment to families of CAZymes as a function of the enzyme's modular composition and significance of similarity. Based on the expert curated CAZy database containing a library of > 100,000 annotated modules.

Functional annotation designed to "age well". Designed to survive experimental characterization, our functional annotations avoid over-predicted subtract / product specificity. It is based on a gradient as a function of similarity to characterized hits. Only 100% identity to a traceable experimentally characterized hit allows assignment of an E.C. number in CAZy.



Example:
in a family that contains β-mannosidases, β-galactosidases and β-glucuronidases, all enzymes hydrolyze equatorially oriented glycosidic bonds. A strong similarity to β-galactosidases allows annotation as "candidate β-galactosidase", but if similarity is not sufficient for a safe prediction of substrate specificity, the best possible annotation is "candidate β-glycosidase". If the similarity is even weaker the best possible annotation would be "related to β-glycosidase".

FUNGAL CAZomes

> 155,000 **ORFs** scanned against CAZy libraries.
> 4,700 genes encoding putative **CAZymes**, including:

~ 2,600	**GHs**	covering	63 families
~ 1,300	**GTs**	covering	35 families
~ 450	**CBMs**	covering	15 families
~ 310	**CEs**	covering	9 families
~ 105	**PLs**	covering	8 families

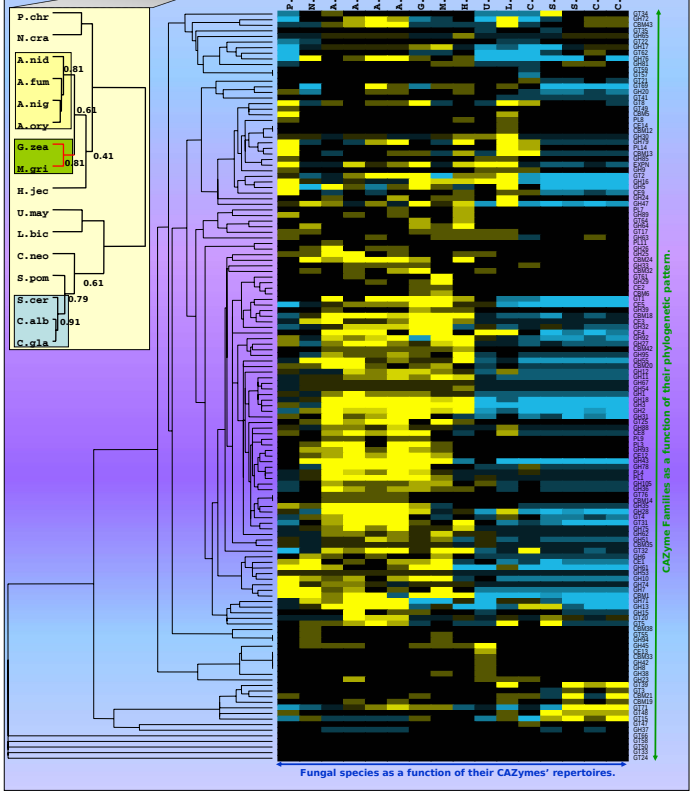
On average, **3% of a Fungal Genome** (among the **higher rates** in eukaryotes)

Wide variations from ~2 % in Saccharomycotina to ~ 4 % in Aspergilli.

 # GHs > # GTs in Fungi as **opposed** to other Eukaryotes.

Wider variations of the **GH** repertoires (SD ~4.25) than the GT's (SD ~2.93). **More** universally **conserved GT** families than **GH** families.

Double hierarchical clustering:



CAZyme repertoires vary both as a function of the **phylogenetic distance** and as a function of **lifestyle / ecotype**.

The **GH families**, a significant proportion of which are **secreted**, show **higher variations** and seem to be more subject to **adaptations** to the environment. Polysaccharide Lyases (**PL**) follow the **same trend**.

In contrast, the **GT** repertoires appear more "**housekeeping-like**" and their divergence reflects more the phylogenetic distance than the lifestyle.

Consistent with previous observations suggesting that **protein secretion is coupled to higher evolutionary rates** [1] (at amino-acid substitution level).

[1] Luz H, Vingron M: **Family specific rates of protein evolution**. *Bioinformatics* 2006, **22**(10):1166-1171.

	Proteome size	GH	%GH	GT	%GT	CBM	PL	CE	% CAZymes
A.nid	9 541	247	2.59	89	0.93	34	19	28	4.01
A.fum	9 926	260	2.62	95	0.96	51	13	28	3.99
A.nig	14 165	240	1.69	111	0.78	35	8	23	2.70
A.ory	12 074	283	2.34	110	0.91	29	21	27	3.65
M.gris	11 109	229	2.06	87	0.78	56	4	47	3.30
N.cra	10 079	169	1.68	71	0.7	38	3	21	2.62
H.jec	9 997	193	1.93	87	0.87	35	3	15	2.98
G.zea	11 640	237	2.04	95	0.82	60	20	42	3.38
C.alb	6 419	58	0.9	69	1.07	4	0	3	2.03
S.cer	6 294	45	0.71	67	1.06	9	0	3	1.83
C.gla	5 283	38	0.72	73	1.38	10	0	3	2.16
S.pom	4 824	48	1	61	1.26	5	0	5	2.36
C.neo	6 572	75	1.14	62	0.94	9	3	9	2.27
L.bico	21 244	163	0.77	83	0.39	24	7	19	1.28
P.chr	11 777	179	1.38	65	0.7	44	4	16	2.24
U.may	6 522	101	2.74	71	1	8	1	20	2.96

Ascomycetes

Richest sets of **degrading** CAZymes (GHs, PLs, CEs) in **Versatile Saprophytes** and **Phytopathogens**

Relatively "**constant**" sets of **GTs**

Basidiomycetes

~ **Rich** sets of **degrading** CAZymes (GHs,CEs) in a **Saprophyte**, a **Symbiont**, and a Phytopathogen (CEs only)

"**Constant**" set of **GTs**

Variable set of **CBMs**

Paucity of **PLs**

Work in Progress: Identification of CAZyme families that underwent significant **expansions / reduction during evolution**. Collaboration with Matthew Hahn, University of Indiana [1,2]. Early results confirm that **GH families evolve faster** than GT families. We identified several CAZyme families in each class that significantly expanded or reduced in specific lineages.

[1] Hahn MW, De Bie T, Stajich JE, Nguyen C, Cristianini N: Estimating the tempo and mode of gene family evolution from comparative genomic data. Genome Res 2005, 15(8):1153-1160.
[2] De Bie T, Cristianini N, Demuth JP, Hahn MW: CAFE: a computational tool for the study of gene family evolution. Bioinformatics 2006, 22(10):1269-1271.

Future directions:

- **Classify species** based on **subset** of CAZymes families and subfamilies known to contain members **involved** in activities of **industrial** or **biological interest** (pectinolytic, cellulolytic, …)
- Correlate **clustering** of fungal **species** based upon **CAZyme repertoires** to actual **experimental** biological **data** related to their ability to degrade various substracts and other biological features.

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