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Enzyme Profile Relatedness analyzed for *Aspergillus* species

Lene Lange

BioEconomy, *Research & Advisory*, Denmark

SME-Research Partner to Danish, Nordic, European and International Research Projects

Co-authors:

Kristian Barrett, Jens C Frisvad, Anne S Meyer

Technical University of Denmark

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The global perspective of Enzyme discovery:

Fungi can play a prominent role for improved use of global biological resources => Need for many new & improved enzymes!

- Fungal invasive power enables upgrading of bioresources now wasted
- Species of *Aspergillus* have a rich biomass conversion capacity
- Even more importantly, *Aspergillus (& Penicillium)* are excellent secreters
- Non-toxin producing *A.spp & P. spp* can be used for producing enzyme blends for biomass conversion => more food & feed from residues, side-streams & wastes

Lange & O´Connor et al. 2021: *Developing a Sustainable and Circular Bio-Based Economy in EU. Front. Bioeng. Biotechnol.*

Elucidating EVOLUTION of fungal enzyme secretome

New functional annotation approach is needed:

Integrate Protein-Family & EC-Function into one ”Function;Family Observation” ”F;F”

Secretome composition of ”F;F” is target for Evolutionary pressure:

- **Fitness: Having the needed types of digestive functions in right types of proteins**
  - *Protein structure determines substrate accessibility, stability, pH- and temperature- tolerance and optimum (etc)*

Annotated ”Function;Family” profiles mimick evolutionary fitness:

  - Optimized secretome mobilizes the substrate efficiently, hereby giving basis for fitness in growth and reproduction

Barrett, Jensen, Meyer, Frisvad*, Lange*, 2020*: Fungal secretome profile categorization of CAZymes by function and family corresponds to fungal phylogeny and taxonomy: Example Aspergillus and Penicillium. Scientific Reports*

Enzyme Profile Relatedness, EPR

-A new, cross taxonomy relevant method, based on ”F;F” annotation:

1. Based on CUPP* functional annotation, establishing the ”F;F” profile of a species

2. Enabling binary ”F;F” profile-comparisons, establishing secretome relatedness

-converting the ”F;F”Observations/Species matrix to a Distance clustering

*CUPP, Conserved Unique Peptide Patterns for automated CAZyme functional annotation

Barrett, K and Lange, L, 2019*: Peptide-based functional annotation of carbohydrate-active enzymes by conserved unique peptide patterns (CUPP). Biotechnol Biofuels (2019) 12:102*

Barrett, K, Hunt, CJ, Lange L, and Meyer, AS, 2020*: Conserved unique peptide patterns (CUPP) online platform: peptide-based functional annotation of carbohydrate active enzymes. Nucleic Acids Research, 2020, Vol. 48.* ***Available online! Try it!***

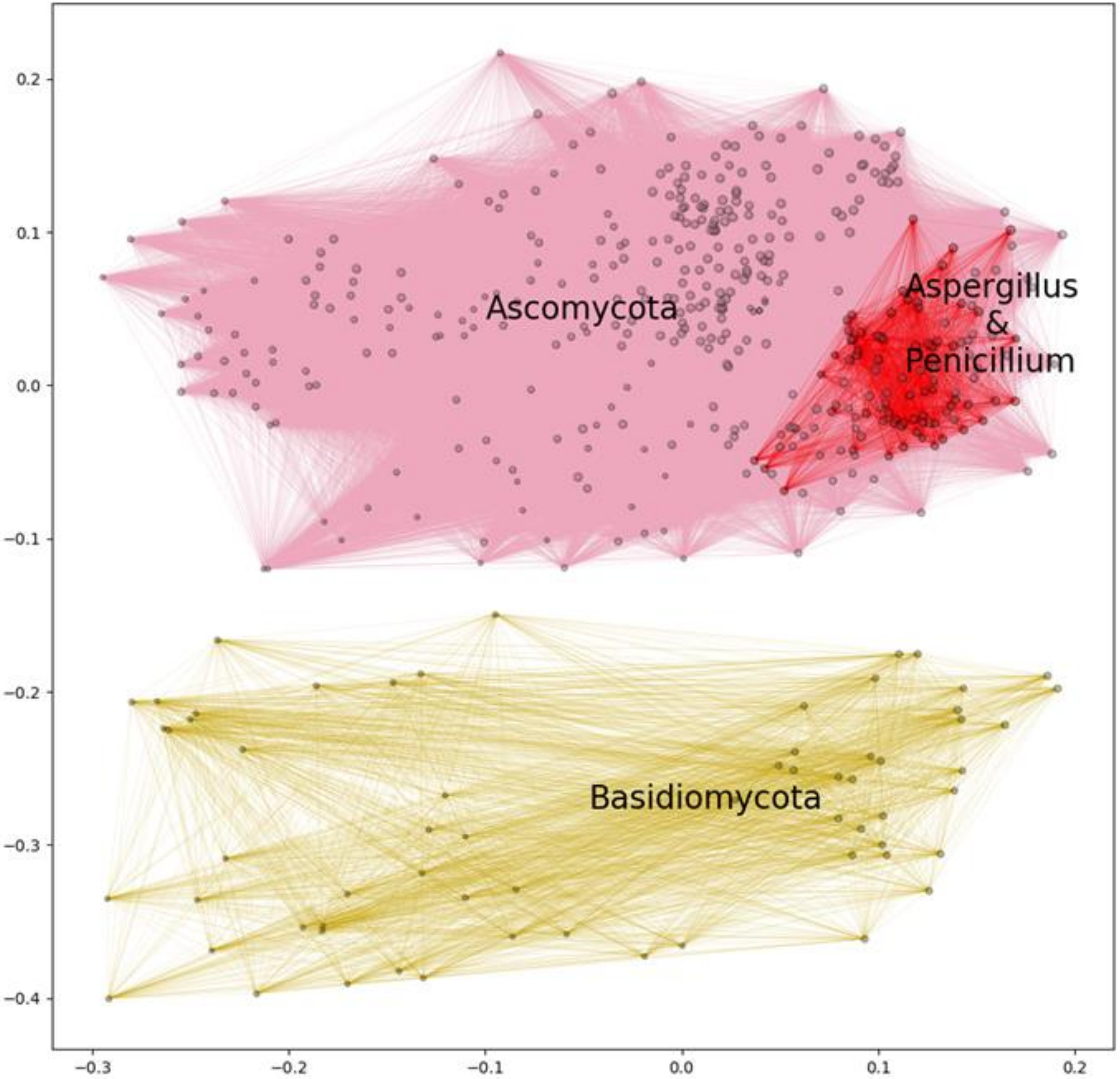
”F;F”-based Enzyme Profile Relatedness

By Multi-Dimensional-Scaling:

***Distinct separation of Asco- from Basidiomycota!**

Distinct clustering of *Aspergillus- & Penicillium spp

Barrett, Jensen, Meyer, Frisvad, & Lange, 2020: *Fungal secretome profile categorization of CAZymes by function and family corresponds to fungal phylogeny and taxonomy: Sci Rep 10, 5158*



List of EPR observations, omitted from EPR-MDS and EPR-dendrogram:

CAZyme “F;F” observations found in all parts of Asco- and Basidiomycota (e.g. fungal cell wall modifying enzymes)

Observation	Substrate	A	B
AA1:1.10.3.2	Laccase	99	100
AA3:Unknown	General	98	95
AA9:1.*.*.*	LPMO	94	53
AA11:1.*.*.*	LPMO	100	31
CE4:Unknown		87	72
GH3:3.2.1.21	Cellulose	99	98
GH5:3.2.1.4	Cellulose	90	81
GH7:3.2.1.176	Cellulose	82	55
GH12:3.2.1.151	Xyloglucan	61	14
GH16:2.4.1.*		93	0
GH16:3.2.1.*		93	0
GH16:3.2.1.39		95	73
GH17:Unknown		100	2
GH17:2.4.1.*		40	0
GH18:3.2.1.14	Chitin	98	58
GH31:3.2.1.20	Starch	85	83
GH43:Unknown		87	47
GH43:3.2.1.99	Pectin	71	30
GH72:2.4.1.*		100	84
GH76:3.2.1.101		100	38
GH92:Unknown	Cellulose	79	66
GH132:3.2.1.*		95	0
PL1:4.2.2.10	Pectin	63	14
PL4:4.2.2.23	Pectin	64	45

Hypothesis:

EPR Clustering, based on ”F;F” annotated digestive profiles matches organismal phylogeny

Validation model:

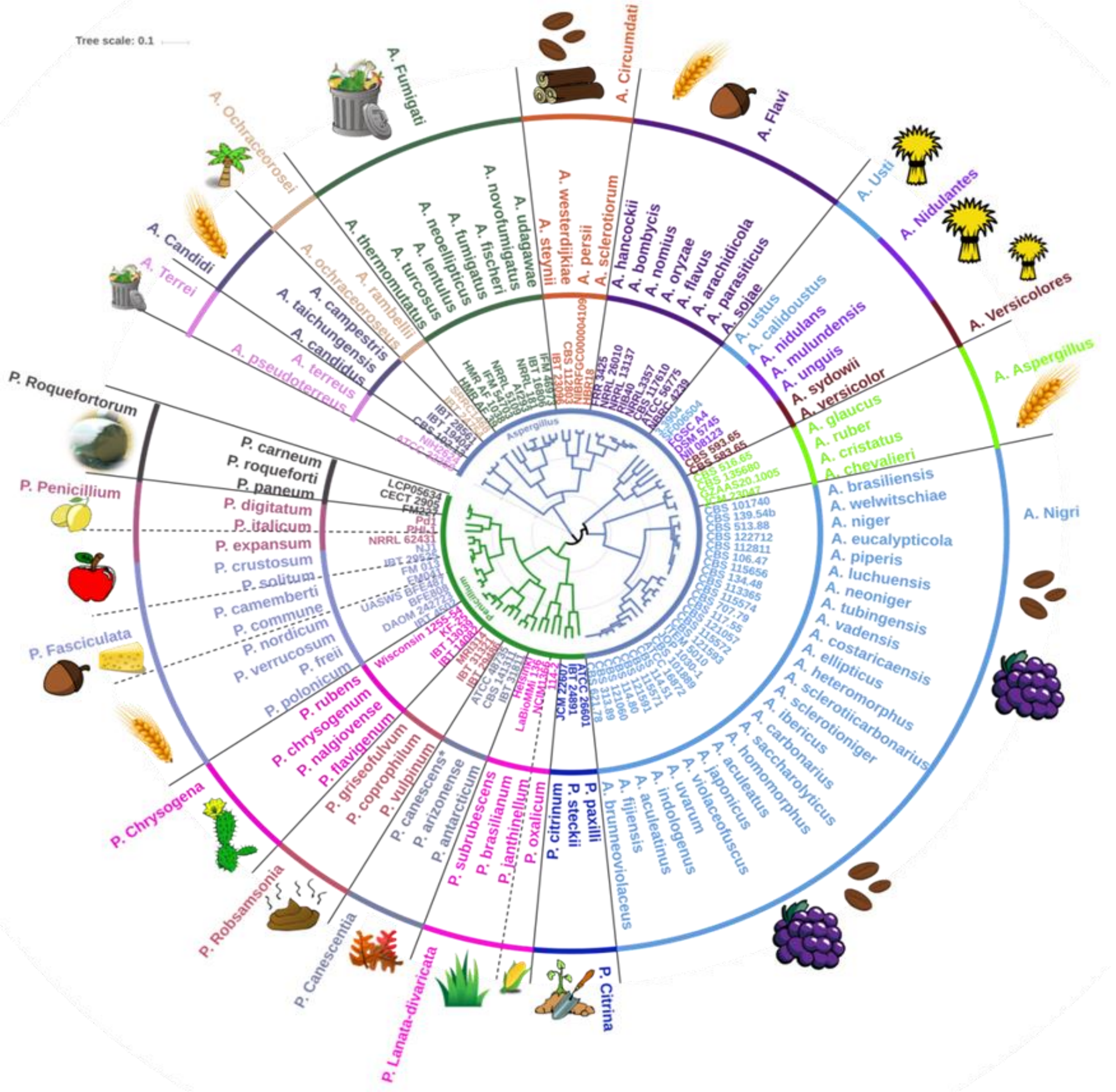
All available genome sequenced *Aspergillus* and *Penicillium*

- Testing by Jacquard calculation: Did not confirm hypothesis!
- **Testing by Yule distance calculation confirmed the hypothesis!**
  - ***Yule** gives equal weight to F;F observations **shared** & F;F observations **shared** NOT having (presence and absence)*

Barrett, Jensen, Meyer, Frisvad, Lange*, 2020: Fungal secretome profile categorization of CAZymes by function and family corresponds to fungal phylogeny and taxonomy: Example Aspergillus and Penicillium. Scientific Reports*

New, across taxonomy, functional genome annotation, EPR

Binning
Functional Genomics to Organismal phylogeny & Interaction-phenotype (=substrate affinity)



Digestive Capacity and Function Diversity in *Aspergillus* Sections

Highest Cellulose degrading capacity		
Section	Total Obs.	Unique Obs.
A. Fumigati	152	135
A. Circumdati	153	134
A. Flavi	159	140
A. Nigri	169	148
P. Lanata-devaricata	161	137

Lowest Cellulose degrading capacity		
Section	Total Obs.	Unique Obs.
A. Ochraceorosei	79	76
A. Aspergillus	99	94
P. Roquefortorum	103	100

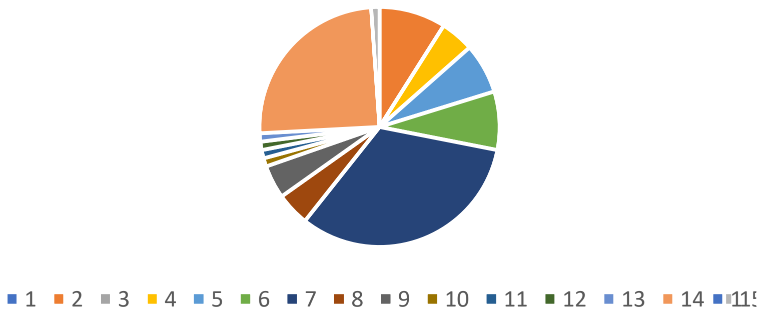
HOT-SPOT analysis, summing-up CAZyme ”F;F” observations:

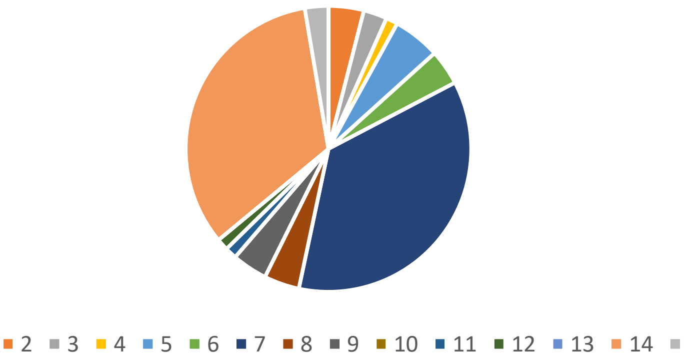
Top-scoring species of *Aspergillus* (”F;F”, on Cellulose+Xylan+Pectin+Lignin)

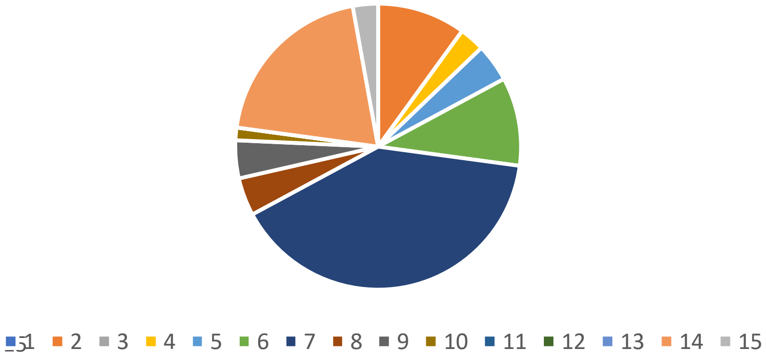
Name	Total	Unique
Aspergillus latus	342,5	85
Aspergillus sp. MA 6041	293	96
Aspergillus calidoustus	254,5	88
Aspergillus sergii	226	89
Aspergillus ustus	224	89
Aspergillus sojae	223	86
Aspergillus sp. ATCC 12892	217	86
Aspergillus versicolor	216,5	98
Aspergillus novoparasiticus	215,5	86
Aspergillus pseudocaelatus	215	83
Aspergillus amoenus	214,5	96
Aspergillus caelatus	213,5	81
Aspergillus sp. MA 6037	212	95
Aspergillus sydowii	210	95
Aspergillus transmontanensis	210	84

Same set of CAZy ”F;F” Obs, from highest capacity to lowest redundancy

	Species Name	GH5;3.2.1.4	GH12; 3.2.1.4	GH6;3.2.1.91	GH7; 3.2.1.176	GH1; 3.2.1.21	GH3; 3.2.1.21	AA3;Unknown*
Top1	*Aspergillus latus*	8	0	4	6	7	29	22
Top2	*Aspergillus sp MA6041*	3	2	1	4	3	27	25
Top3	*Aspergillus calidoustus*	7	0	2	3	7	28	14
3rd Last	*Aspergillus coremiiformes*	4	0	1	3	1	7	6
2nd Last	*Aspergillus sp HF37*	2	0	1	1	2	9	5
Lowest	*Aspergillus cejpii*	0	1	0	0	0	4	4


Top 1 *Aspergillus latus*
14 Unique, 94 Total


Top 2 *Aspergillus sp* MA6041
13 Unique, 78 Total


Top3 *Aspergillus calidoustus*
12 Unique, 73 Total

***Sützl, L., Laurent, C.V.F.P., Abrera, A.T. et al.* Multiplicity of enzymatic functions in the CAZy AA3 family. Appl Microbiol Biotechnol 102, 2477–2492 (2018)**

Conclusions

- When annotated by ”EC Function;CAZyme Family” integrated observations, Asco- & Basidiomycota cluster separately and *Aspergillus & Penicillium* cluster together
- EPR-dendrogram of digestive secretome relatedness, gives a stunning match to divisions of *Aspergillus & Penicillium* into Sections
  - **Evolution of CAZyme secretome is an integrated part of speciation**
- *Aspergillus* sections with highest digestive capacity are: A. Flavi and A. Nigri.
- Use **CUPP, EPR & Hot-Spot analysis for targeted, efficient enzyme discovery** and for improved blend composition for efficient, feedstock-versatile biomass conversion

Consider to:

- Include digestive enzyme ”F;F” profile in Species description
- Include digestive enzyme ”F;F”profiles in characterization of Sections

Recommended focus:

Not just who the fungi are, but also what they do!

Thanking for the opportunity and attention ☺