BioEconomy
Research & Advisory

From genome sequence to Biology & Applied-relevance

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**Improved use of the Bioresources,
”Circular & Bio-based”, by use of Fungi & Enzymes**

has potentially significant positive impact on:

Climate, Food security, Public Health, Biodiversity, Local Jobs - and Global Business!

For all this **Enzyme Function is key!**

Fungi and fungal enzymes are key for more efficient use of Biological Resources, upgrading what is now lost

Overview of Opportunities for more responsible use of biological resources:

- **Upgraded use** of *food waste;* WHO: 34% food lost globally!
- **Valorization** of *food-processing side-streams =>approx additional >20% wasted*
- **Improved use** of *crop residues –unlocking full potential of the entire crop plant*
- **Sustainable use** of *blue biomass, seaweeds & fish cut-offs; & of forestry waste*
- **Upcycling of *residual microbial biomass*** from biological production (bacteria & fungi)
- **Making value from organic content** of *sludge and wastes*
- **Upgraded use of fibers** from *outsorted textiles, for production of new textiles*

Lange, O´Connor et al., 2021: *Developing Sustainable & Circular biobased Economy in EU. Front.Bioeng.Biotechnol. 2021*

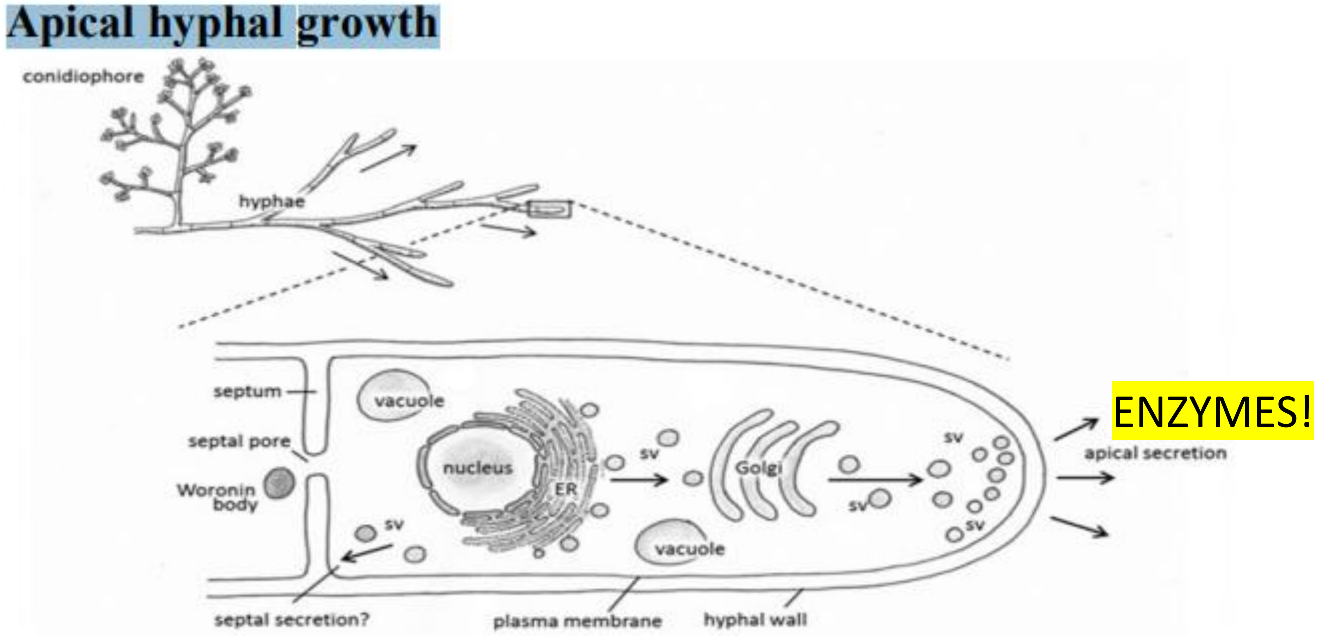
New methods for function-targeted Enzyme Discovery

-opening for conceptually new understanding of secretome evolution

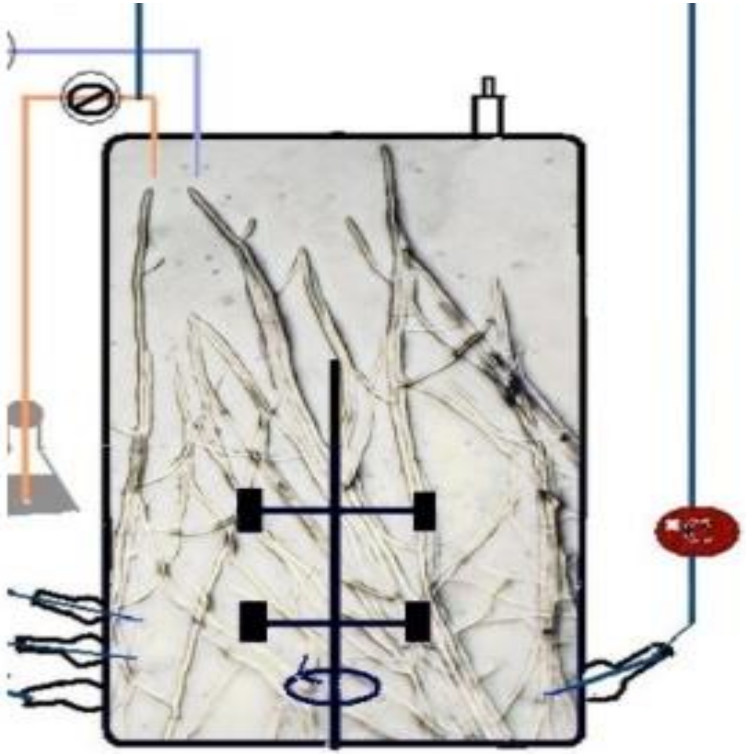
1. CUPP, Conserved Unique Peptide Patterns: Annotating to Family, and cluster enzymes into CUPP groups => Robust function prediction
    - All members of a CUPP group have same function (or share specific function-related features)
2. Integrated genome annotation to ”Function&Family”, is mimicking evolution’s selection for fitness: Having the right function, in optimal type of protein
3. Enzyme profile relatedness, enables comparing secretomes across taxonomy
4. Fungal enzyme HotSpot analysis, identified by summing ”F&F” observations
5. Fungal secretome composition, analyzed by enzyme co-occurrences -*under study*

The invasive power of fungal hyphae

-fungi secretes Enzymes to break down leaves, wood etc = food!
-fungi produce Antibiotics, protecting its ”lunch package” from bacteria*



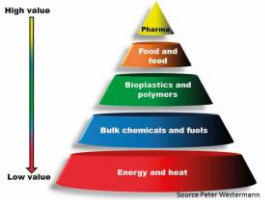
ENZYMES!



Fungi can be grown in fermentors &
Fungi are HotSpot for discovery of new types of enzymes and antibiotics

Sustainability –feeding the world and improved One Health

Unlocking the full potential of the many types of biomass



Source Peter Westermann

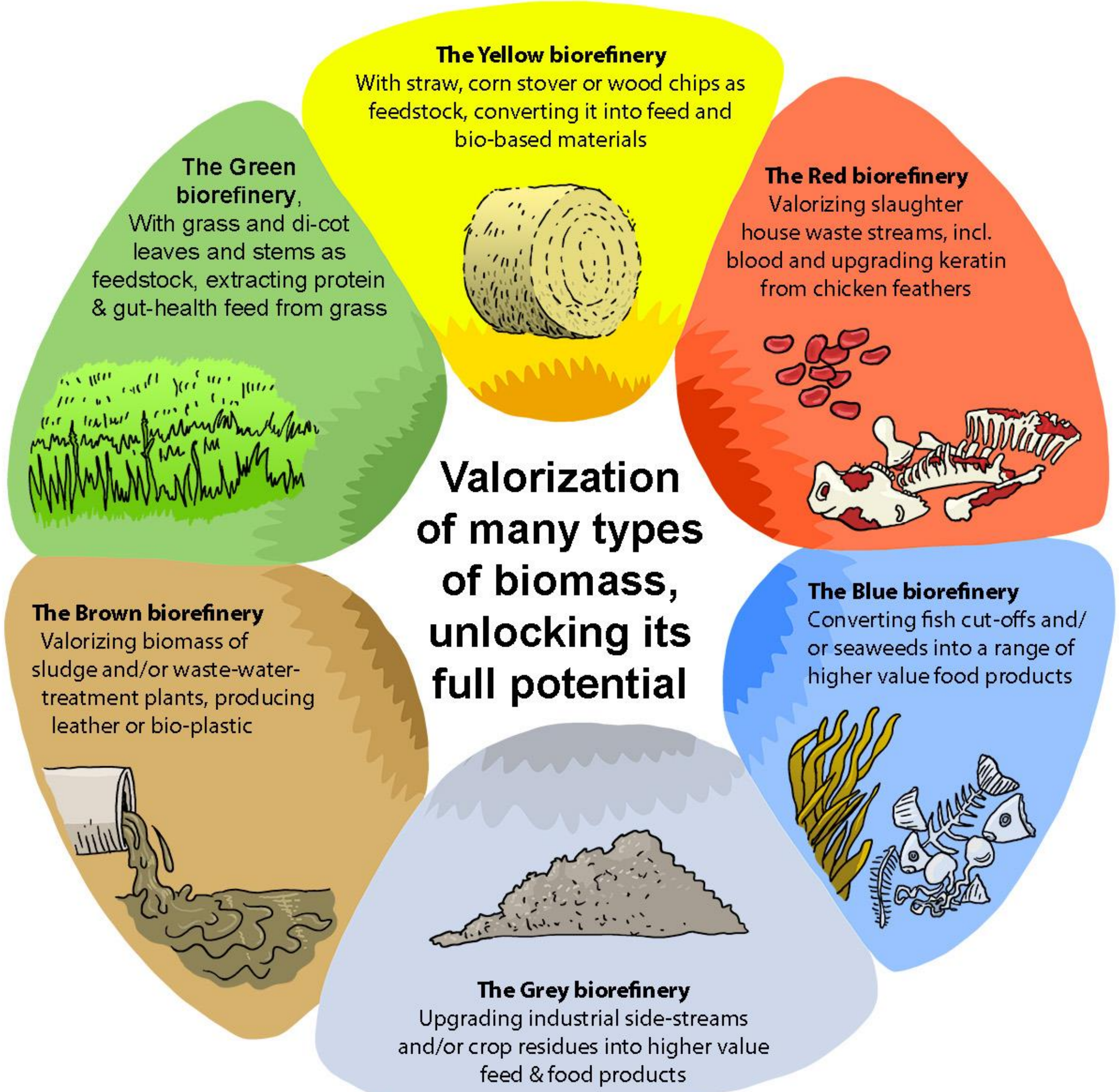
Fungal (& bacterial) enzymes play a significant role for conversion of all types of biomass

- The **Yellow** Biomass: Straw, corn-stover & wood chips
- The **Green** Biomass: Green grass & foliage and stems
- The **Blue** Biomass: Fish processing cut-offs & Seaweeds
- The **Red** Biomass: Slaughterhouse waste, blood & cut-offs)
- The **Grey** Biomass: Gro-industrial side streams)
- The **Brown** Biomass: Sludge & manure; household waste
- The **Purple** approach: Making feed from methane (Negative Emission Tech.)

***Lange et al. 2021:** Developing a Sustainable and Circular Bio-Based Economy in EU. Front. Bioeng. Biotechnol. 2021*
***Lange, L., 2022:** Business Models, including higher value products for the new circular, resource-efficient bio-based industry, Frontiers in Sustainability, in press*

Unlocking full potential of the biomass otherwise wasted

Lange, L., 2022: *Business Models, including higher value products for the new circular, resource-efficient bio-based industry. Frontiers in Sustainability, 2022*



The Yellow biorefinery
With straw, corn stover or wood chips as feedstock, converting it into feed and bio-based materials

The Green biorefinery,
With grass and di-cot leaves and stems as feedstock, extracting protein & gut-health feed from grass

The Red biorefinery
Valorizing slaughter house waste streams, incl. blood and upgrading keratin from chicken feathers

Valorization of many types of biomass, unlocking its full potential

The Brown biorefinery
Valorizing biomass of sludge and/or waste-water-treatment plants, producing leather or bio-plastic

The Blue biorefinery
Converting fish cut-offs and/ or seaweeds into a range of higher value food products

The Grey biorefinery
Upgrading industrial side-streams and/or crop residues into higher value feed & food products

Cascading use is essential, do not only use the energy content

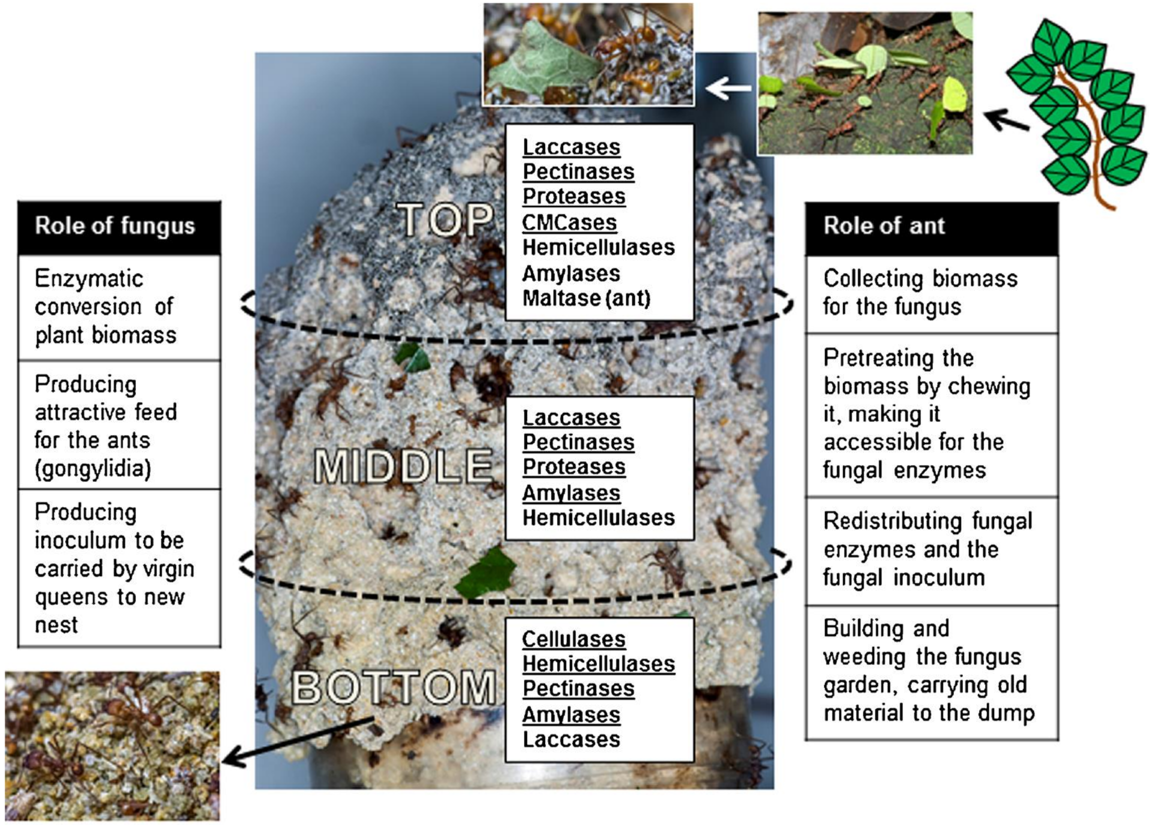
Co-Evolution fungi & insects

= Prototypes of Yellow & Green Biorefinery

Termites growing fungi

The most succesful type of life on earth! Measured in biomass conversion

Leaf cutter ants growing fungi



Role of fungus
Enzymatic conversion of plant biomass
Producing attractive feed for the ants (gongylidia)
Producing inoculum to be carried by virgin queens to new nest

TOP:
Laccases
Pectinases
Proteases
CMCases
Hemicellulases
Amylases
Maltase (ant)

MIDDLE:
Laccases
Pectinases
Proteases
Amylases
Hemicellulases

BOTTOM:
Cellulases
Hemicellulases
Pectinases
Amylases
Laccases

Role of ant
Collecting biomass for the fungus
Pretreating the biomass by chewing it, making it accessible for the fungal enzymes
Redistributing fungal enzymes and the fungal inoculum
Building and weeding the fungus garden, carrying old material to the dump

Denmark is the country in the world who gets most out of Fungi and Fungal products (= >20% of all Danish export of goods!)

Picture: Jens H Petersen

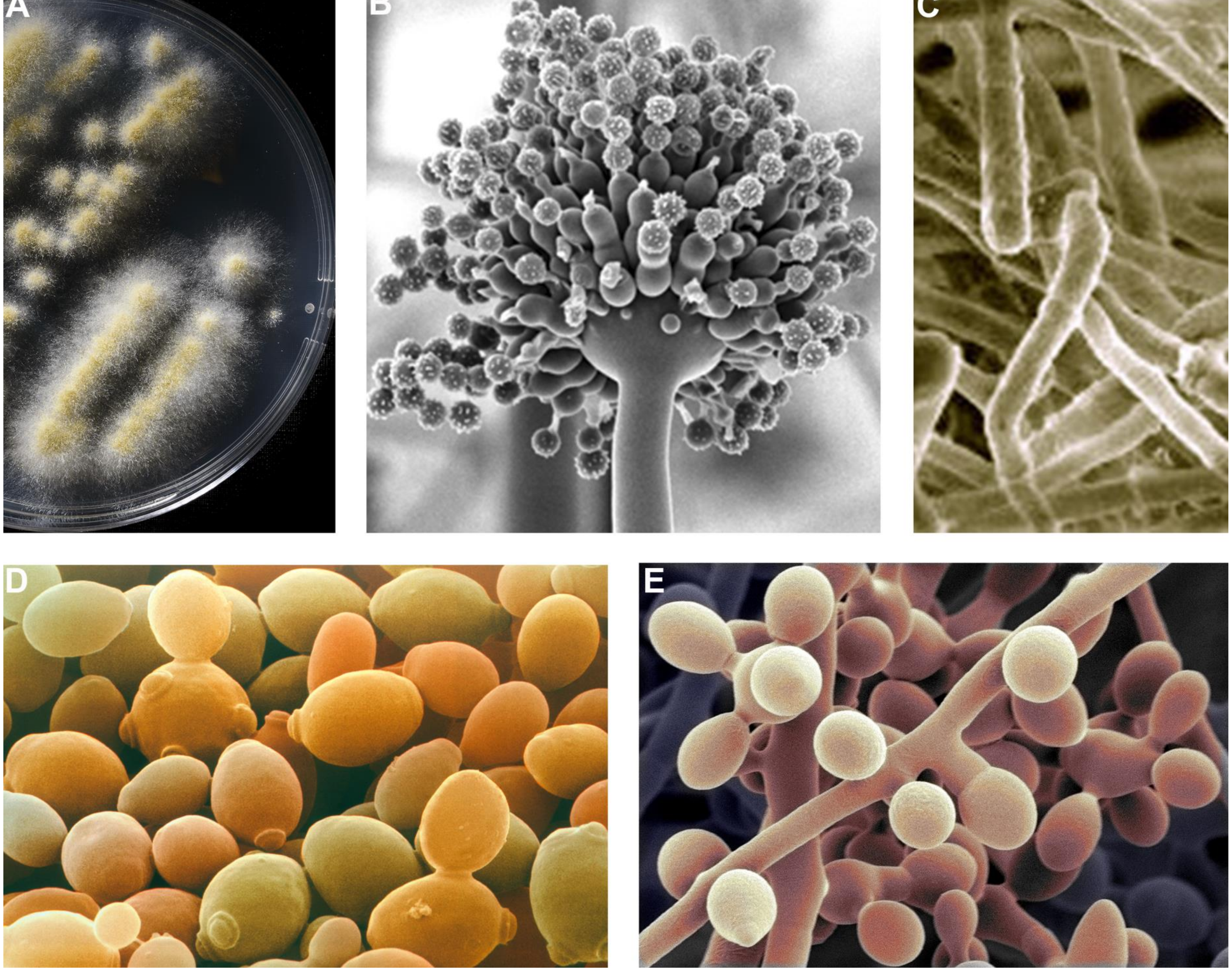
The Fungal Hall of Fame

Biological prodution

Penicellin
Enzymes
Insulin,
Statins

In future?

Meat protein
Milk protein
from yeast!



EUROPEAN
COMMISSION

Brussels, 20.3.2024
COM(2024) 137 final

COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS

Building the future with nature: Boosting Biotechnology and Biomanufacturing in the EU

Developing Peptide-based Functional Annotation

-efficient and robust method, for discovery of enzymes with specific funtions

Background:

- Most annotation is based on alignment of sequences
  - Alignment gives equal weight to all parts of the sequence. Evolution does not!

Alternative, non-alignment-based functional annotation

In evolution, certain parts of gene sequence is conserved for optimized fitness; resulting in shared peptide patterns between groups of different proteins

- **Inventive step 1**: Clustering proteins in groups, sharing same peptide patterns
- **Inventive step 2**: Unique peptide patterns = basis for robust prediction of function!
  - Patent filed 2012, Busk & Lange, Aalborg University
  - Patent placed in public domain 2014: => Peptide-based functional annotation Free to Use for All!

Busk, PK & Lange, L, 2013: *Function-Based Classification of Carbohydrate-Active Enzymes by Recognition of Short, Conserved Peptide Motifs. Appl Environ Microbiol. 79: 3380–3391.*

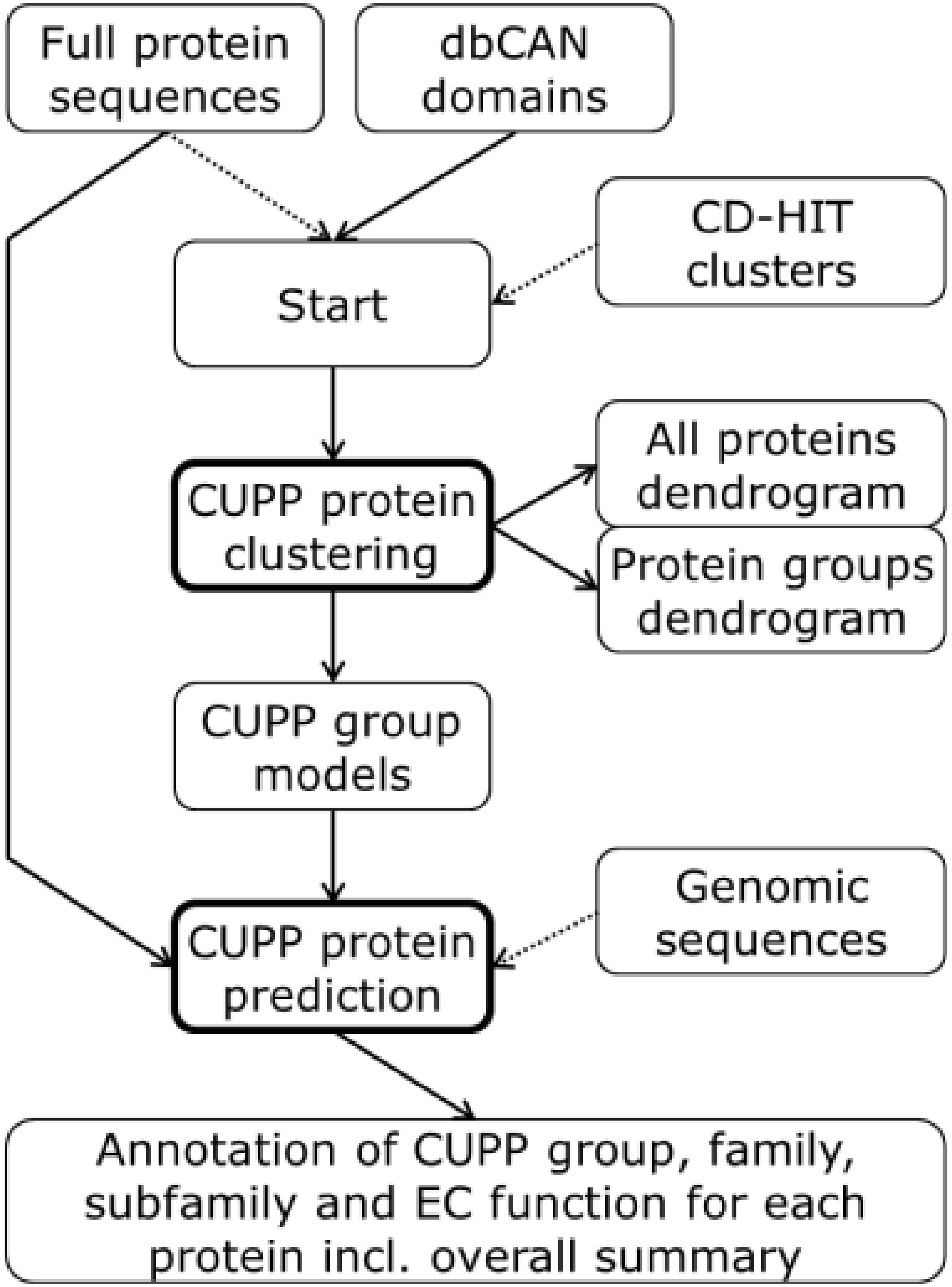
Functional annotation by CUPP

CUPP for CAZymes:

Consists of two stages,
Clustering and *Prediction*

Barrett & Lange, 2019
in Biotech f Biofuel:
CUPP, Peptide-based Functional annotation

Barrett et al., 2020
CUPP as Online functional annotation platform.
Nucleic Acid Research, 48, p110-115



Conserved Unique Peptide Patterns

CUPP, Optimized peptide-based prediction of function

- All groups formed simultaneously => Group #1 no longer disproportionally large
- CUPP Group numbers conserved; allowing for comparing annotation results over time
- Using Unique peptides for each CUPP group –no overlap
- Improved Sensitivity and Precision in Prediction of Function
- Basic principle covered by same, open access patent (PPR)
- CUPP available as automated online platform

- ***Barrett, K & Lange, L, 2019****: Peptide-based functional annotation of carbohydrate-active enzymes by conserved unique peptide patterns (CUPP). Biotechnology for biofuels 12 (1), 1-21. CUPP method, description and validation*
- ***Barrett, K et al., 2020****: Conserved unique peptide patterns (CUPP) online platform: peptide-based functional annotation of carbohydrate active enzymes, Nucleic Acids Research, 48, p110-115.* ***Try it! It is online!***

Annotation to **CAZyme Family, Subfamily & CUPP group**

Validation of CUPP, Case: CAZyme Family GH30

*CUPP groups represent a lower-level division of (sub)families

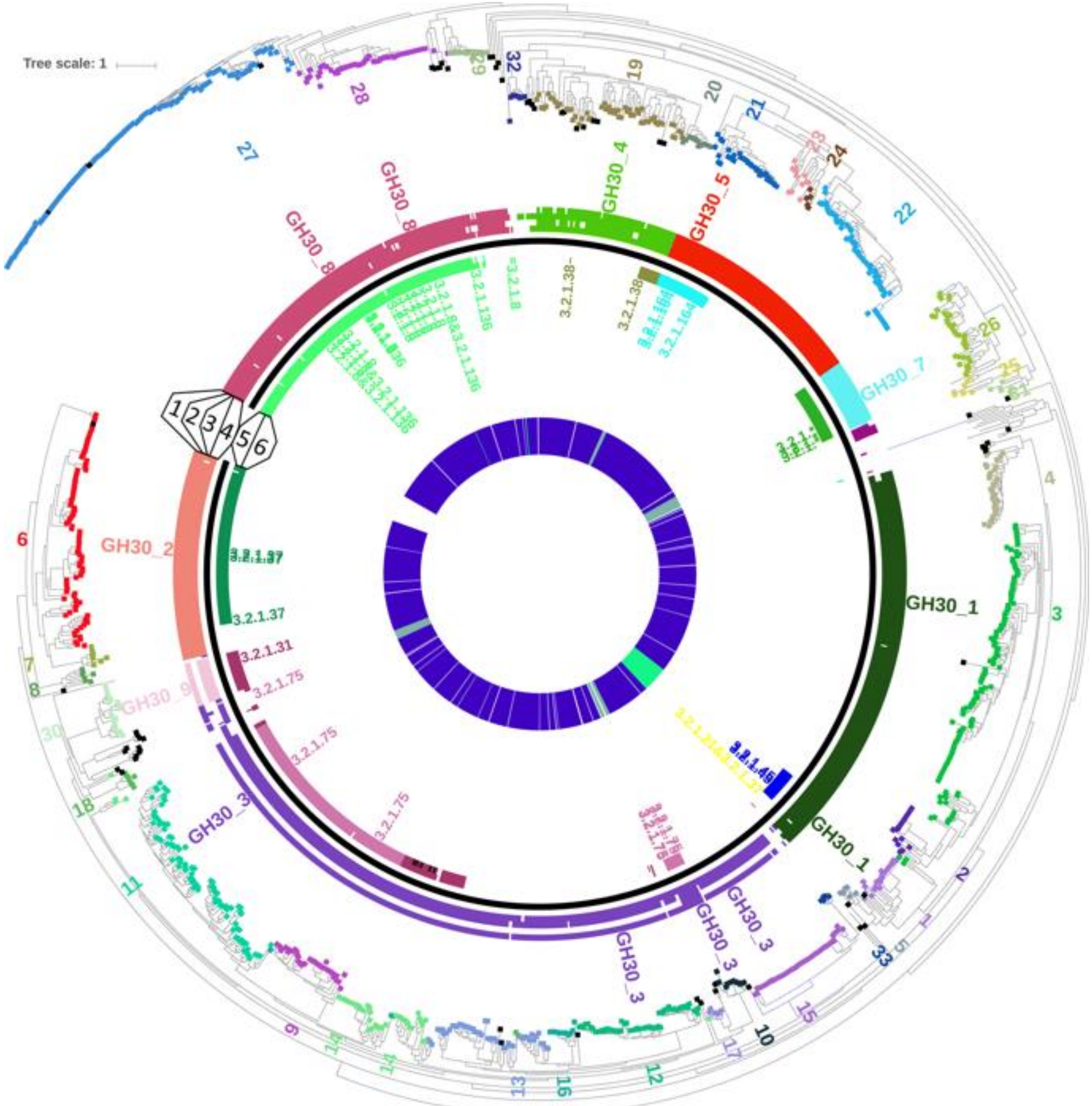
- Each Family / Subfamily hold several functions
- All CUPP groups fall within specific CAZy Family/Subfamily

CUPP grouping, gives basis for Functional Prediction also of uncharacterized proteins:

-All members of a CUPP group have same Function =>

-Prediction of function possible, if just one CUPP group member is characterized

CUPP



CAZy Family GH30:

Multicolored ring:

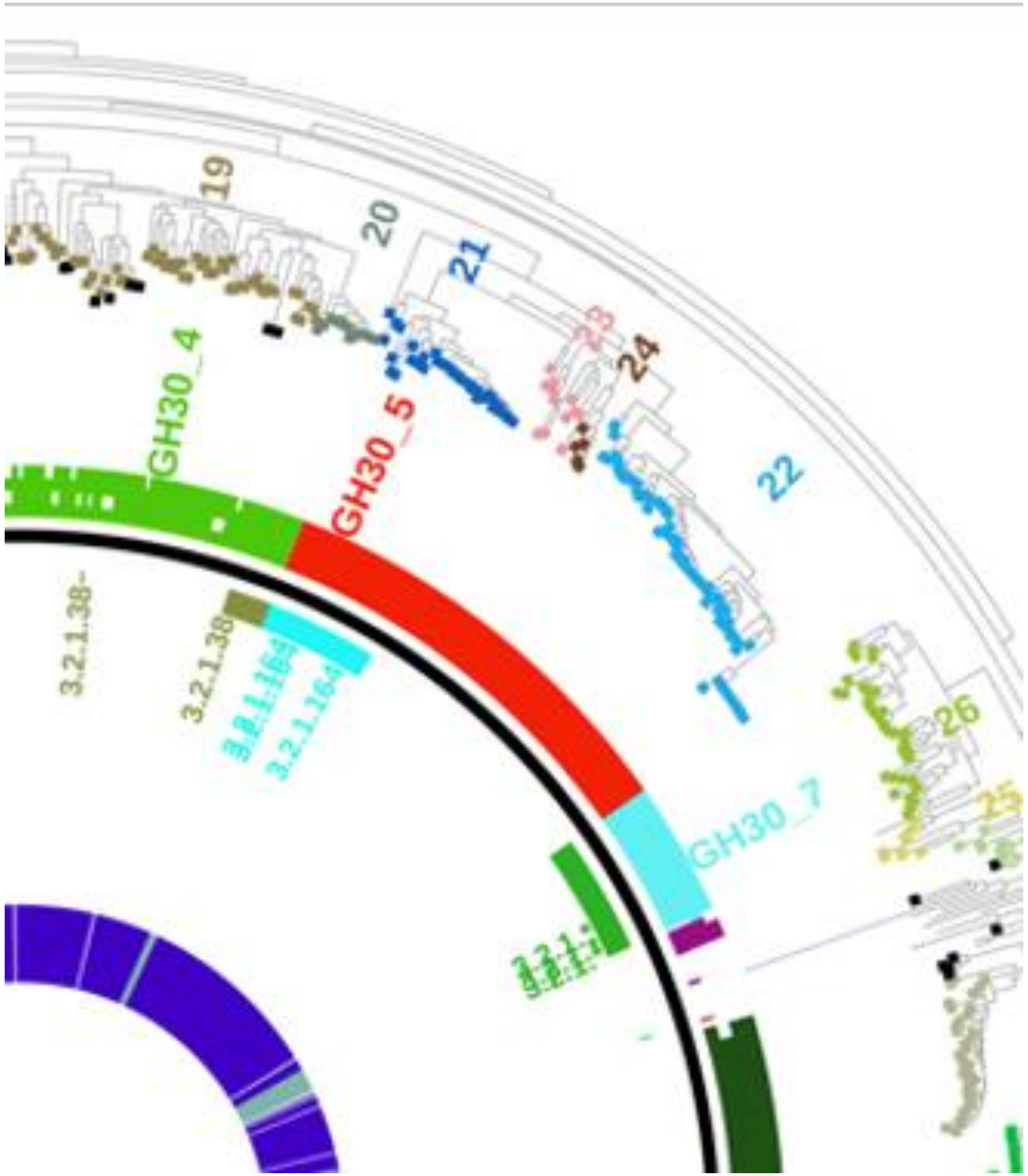
Families, Sub-Families

and EC Functions

The surrounding dendrogram:

CUPP-groups

CUPP



GH30_5: CUPP group 21-24

CUPP 21: EC3.2.1.164
For new enzymes of this CUPP
Group you now know its function

CUPP 22, 23 & 24:
No characterized enzymes!
-if your new enzyme is here
you can characterize it and at the
same time add a new function to
GH30_5

Integrated ”Function;Family” genome-annotation: Mimicks Evolution selection for fittness

F;F

1. Evolutionary pressure favours, having optimized digestive functions, in optimal type of protein-structure:

- Secretome blend of functions determines digestive capacity
- Protein structure determines substrate accessibility, stability, pH- and temperature- tolerance and optimum (etc)

2. ”Function;Family” annotation enables comparing secretome profiles across taxonomies, (binary: having specific F;F observations or not)

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

CUPP & ”F;F”:
EPR analysis of genome sequenced fungal species

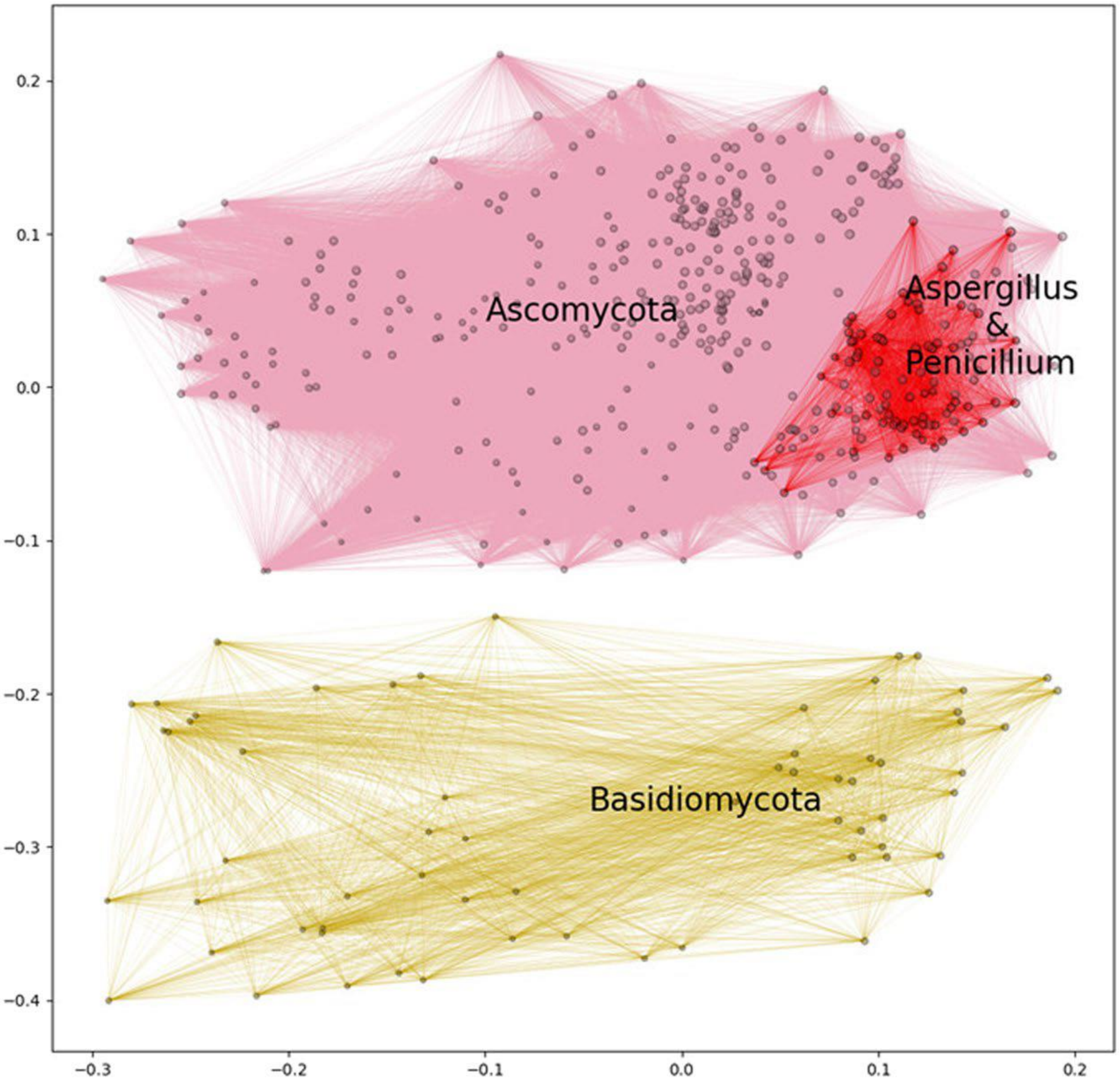
Result:

- 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



Use CUPP-based ”EPR” method for cross-taxonomy comparison of secretome relatedness

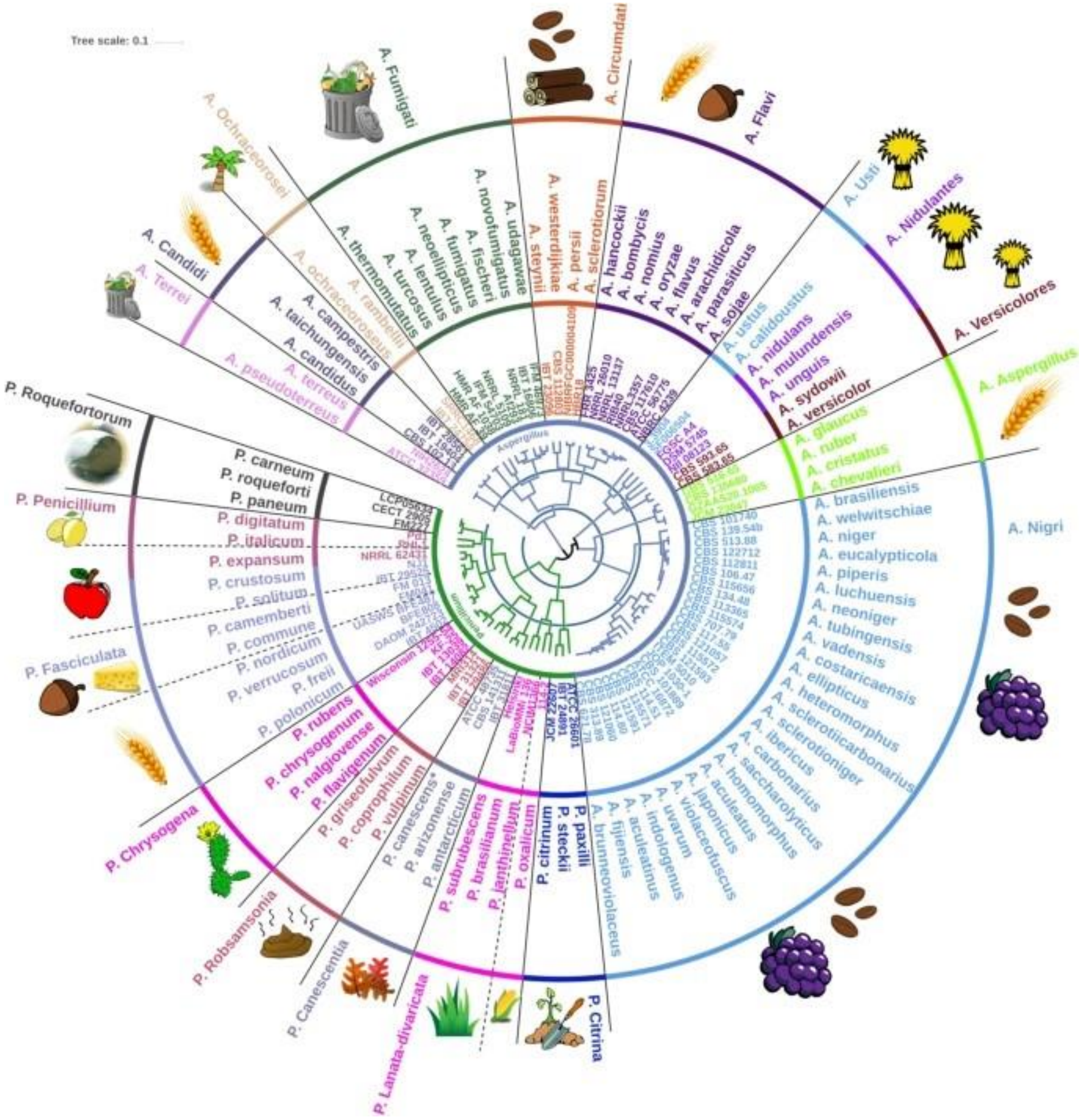
- **Hypothesis:** Phylogenetic relationship of organisms (=phylogenetic trees) match dendrogram based on enzyme profile relatedness (EPR)

  Validation model chosen: All species of *Aspergillus* and *Penicillium*

- Testing hypothesis by *Jacquard* calculation: Did NOT confirm hypothesis!
- Testing hypothesis by *Yule* calculation CONFIRMED the hypothesis!
  - *OBS Yule includes both F;F observations shared & F;F observations shared NOT having*

Ref: 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 (IF4.379), DOI: 10.1038/s41598-020-61907-1*

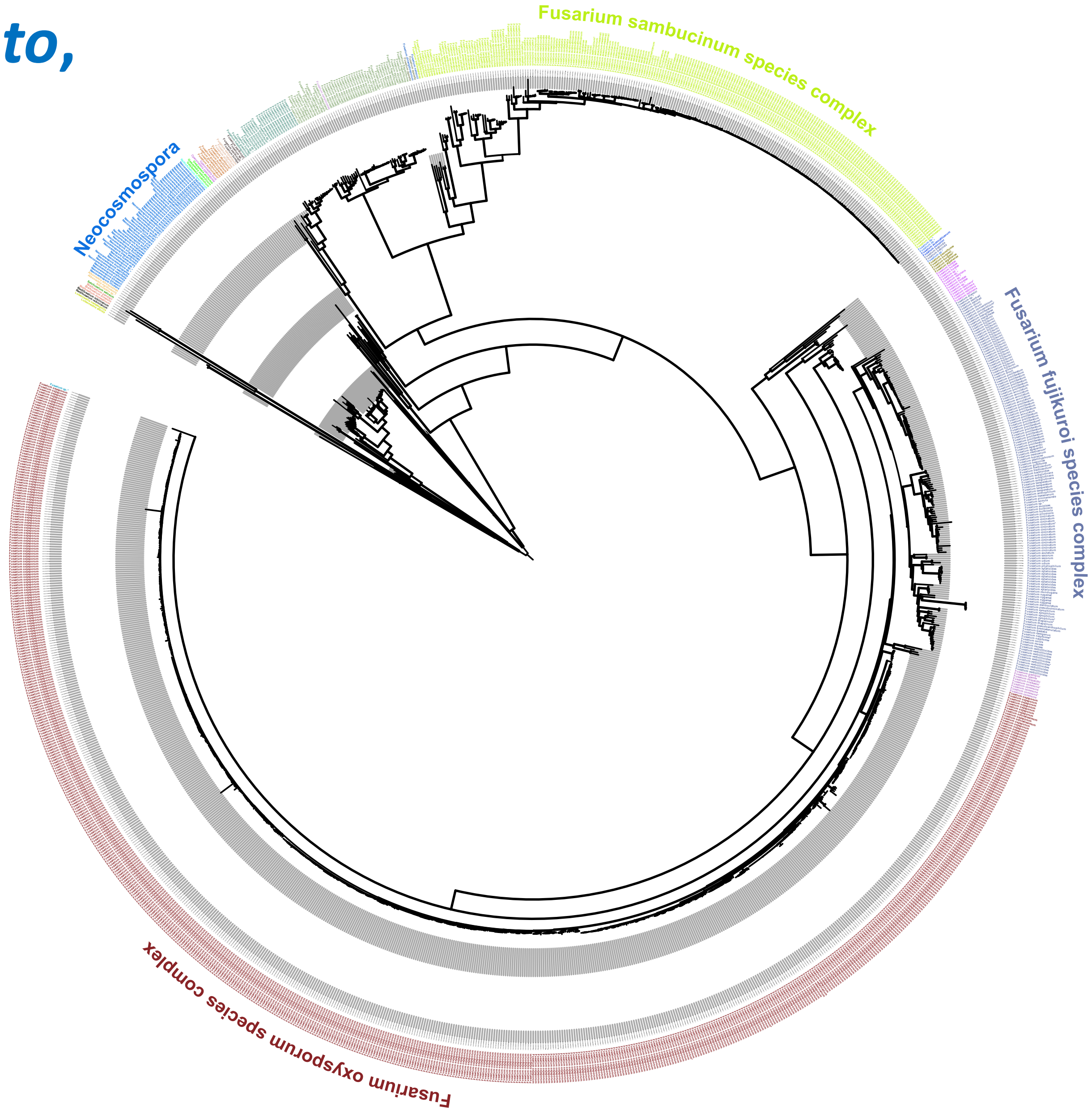
Testing hypothesis on *Penicillium & Aspergillus:* Hypothesis fully confirmed! Dendrogram in center. Total match to all sections in both genera



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 (IF4.379), DOI: 10.1038/s41598-020-61907-1*

EPR

Fusarium sensu lato, Phylogenetic tree



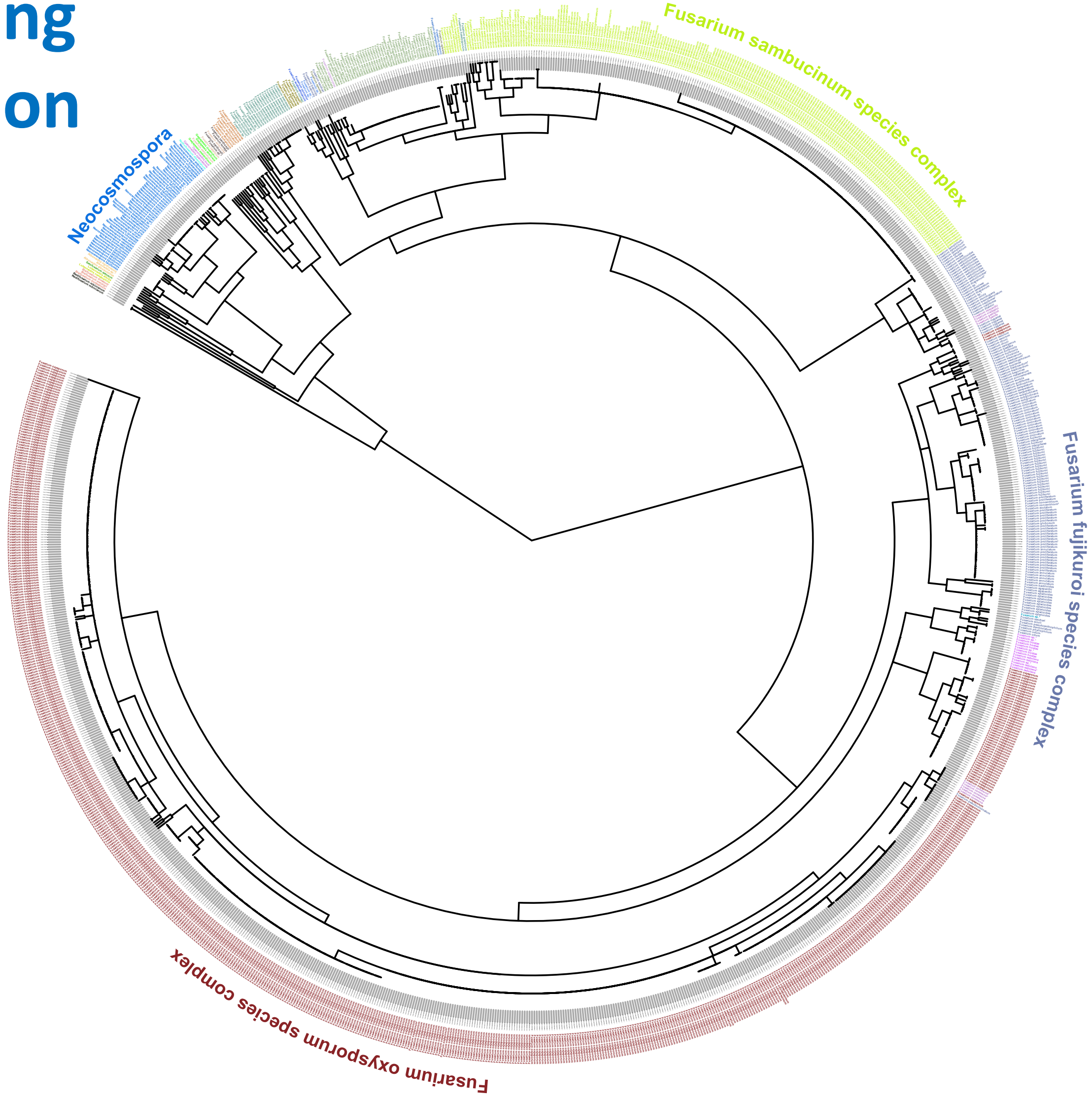
Phylogenetic tree, based on concatenation of 227 single copy orthologs, each representing 99.8% of the genome assemblies.

Resulting in a 82.480 aa multiple alignment; used for construction of a tree by fasttree, displayed using itol.

Lange et al., in prep

Fusarium sensu lato: EPR dendrogram, clustering according to ”F;F” observation relatedness

EPR



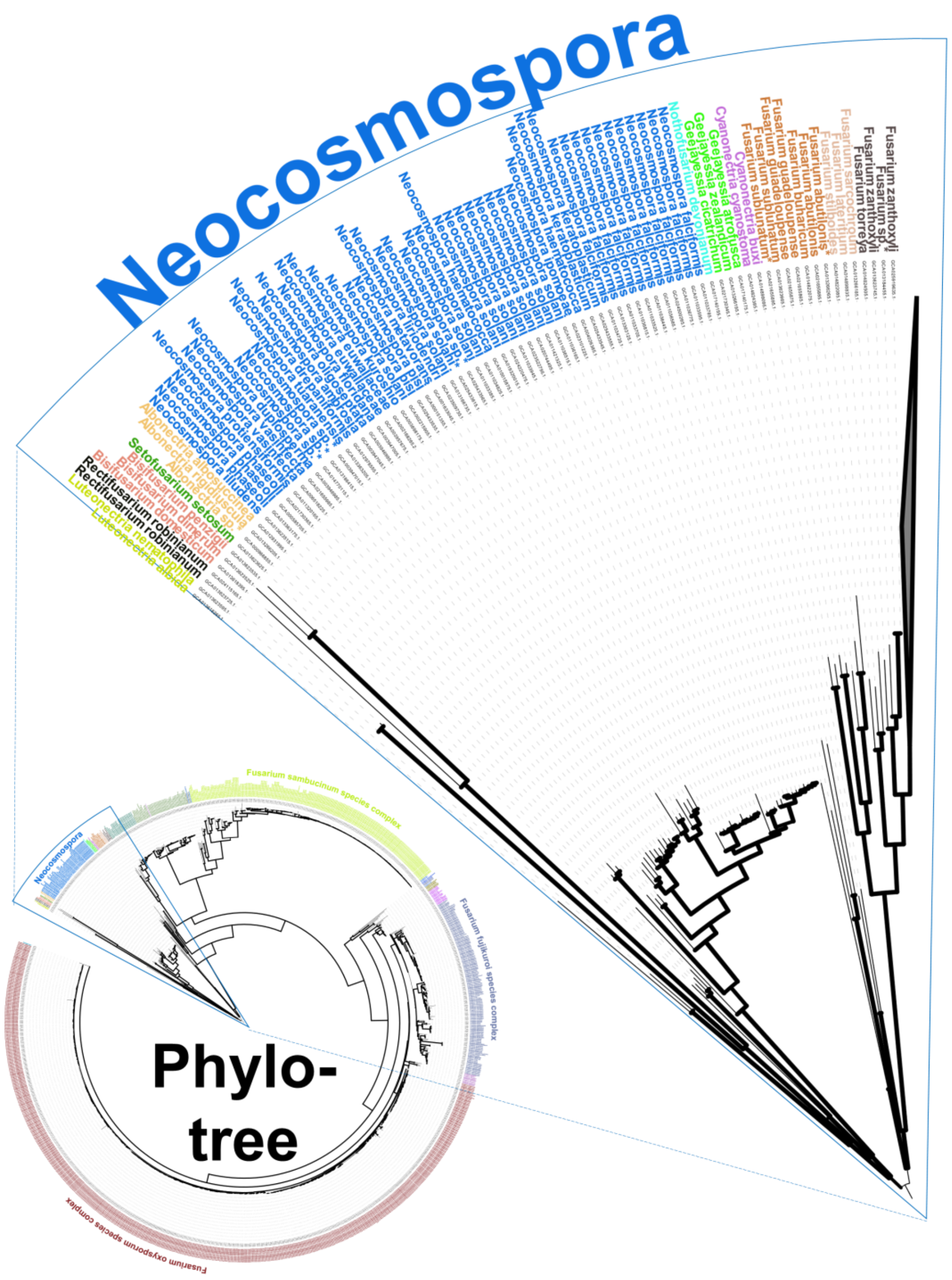
CAZyme EPR dendrogram:

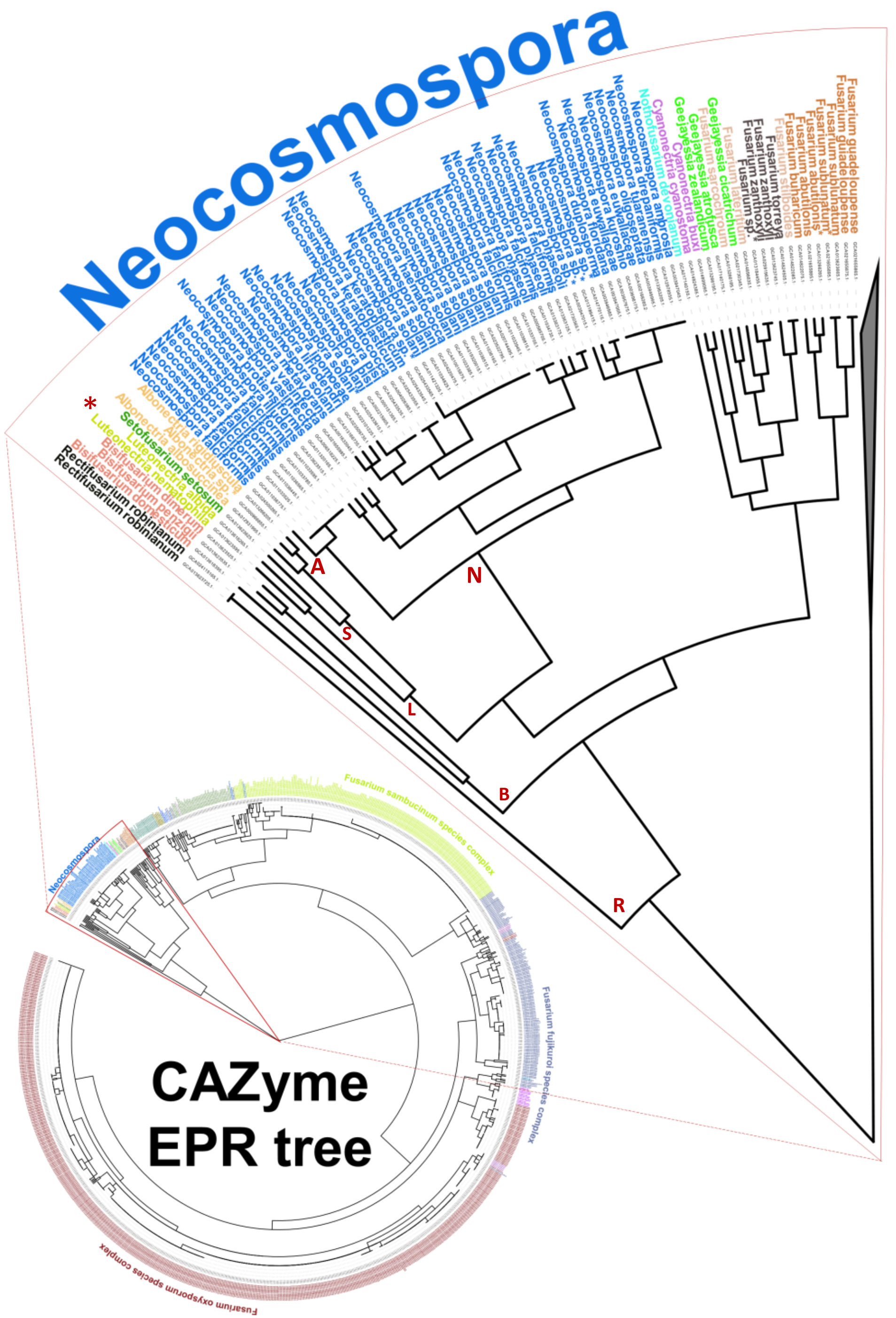
Use CUPP to annotate to family & predict function of secreted CAZymes.

Transform into a dendrogam, where similarity is defined by whether or not individual “function;family”observations are found present or absent in the genome assemblies, using Yule similarity

Lange et al., in prep

EPR


Neocosmospora
Fusarium sambucinum species complex
Fusarium fujikuroi species complex
Fusarium oxysporum species complex
Neocosmospora
Phylo-tree


Neocosmospora
*
A
N
S
L
B
R
Fusarium sambucinum species complex
Fusarium fujikuroi species complex
Fusarium oxysporum species complex
Neocosmospora
CAZyme EPR tree

Hotspot

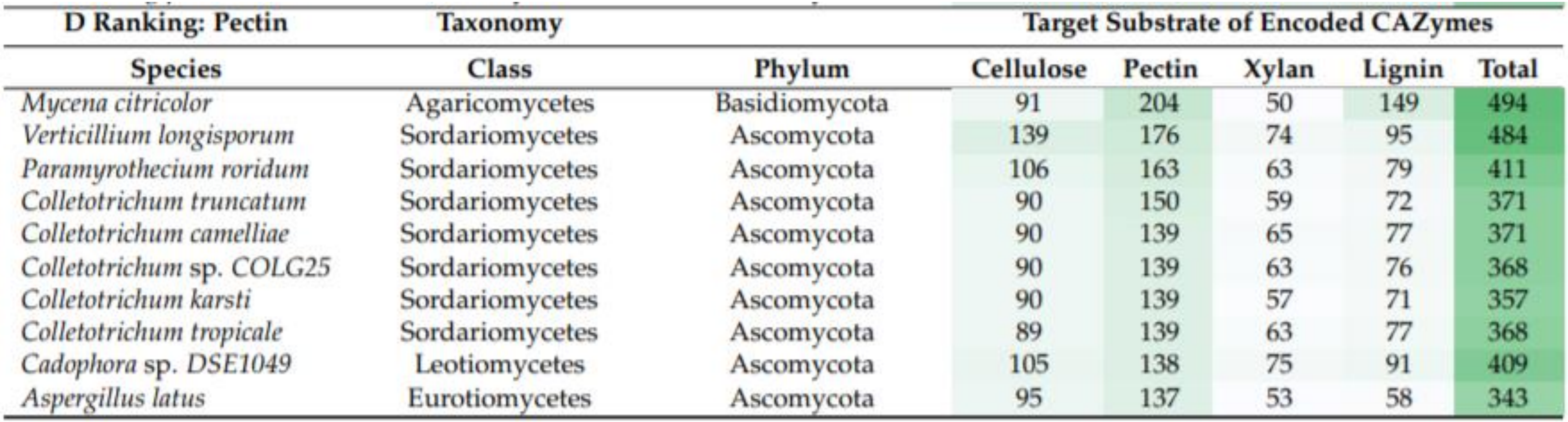
Summing up ”F;F” observations of all genome sequenced fungi

- **Summing up ”F:F” observations per species**, allows for ranking of all Fungal Kingdom genome sequenced species (~2.000), according to:
  - Richness in CAZyme function-specificity diversity (only unique ”F;F” obs.)
  - Total degrading capacity (incl redundant ”F;F” obs.)
- **Analyzing for fungal ”CAZyme Hotspots” identifies**:
  - Fungal species richest in cellulolytic, xylanolytic, pectinolytic & ligninolytic enzyme potential
- **The fungal enzyme Hotspots are found in very different types of fungi**:
  - Many surprises: includes e.g. endophytes and stone-fungi
  - Strongest degrading capacity: rumen fungi

Reference: Lange, L.; Barrett, K.; Meyer, A.S., 2021: *New Method for Identifying Fungal Kingdom Enzyme Hotspots from Genome Sequences. J. Fungi 7, 207*

Hot Spot analysis, example

10 top-scoring fungal species, ranked by pectin-degrading capacity



D Ranking: Pectin	Taxonomy		Target Substrate of Encoded CAZymes				
Species	**Class**	**Phylum**	**Cellulose**	**Pectin**	**Xylan**	**Lignin**	**Total**
Mycena citricolor	Agaricomycetes	Basidiomycota	91	204	50	149	494
Verticillium longisporum	Sordariomycetes	Ascomycota	139	176	74	95	484
Paramyrothecium roridum	Sordariomycetes	Ascomycota	106	163	63	79	411
Colletotrichum truncatum	Sordariomycetes	Ascomycota	90	150	59	72	371
Colletotrichum camelliae	Sordariomycetes	Ascomycota	90	139	65	77	371
Colletotrichum sp. *COLG25*	Sordariomycetes	Ascomycota	90	139	63	76	368
Colletotrichum karsti	Sordariomycetes	Ascomycota	90	139	57	71	357
Colletotrichum tropicale	Sordariomycetes	Ascomycota	89	139	63	77	368
Cadophora sp. *DSE1049*	Leotiomycetes	Ascomycota	105	138	75	91	409
Aspergillus latus	Eurotiomycetes	Ascomycota	95	137	53	58	343

Lange, L, Barrett, K, & Meyer, AS, 2021: *New Method for Identifying Fungal Kingdom Enzyme Hotspots from Genome Sequences. J. Fungi 7, 207*

Mycena citricolor, (Agaricales, Basidiomycota)
-a Hot Spot for pectin degrading CAZymes

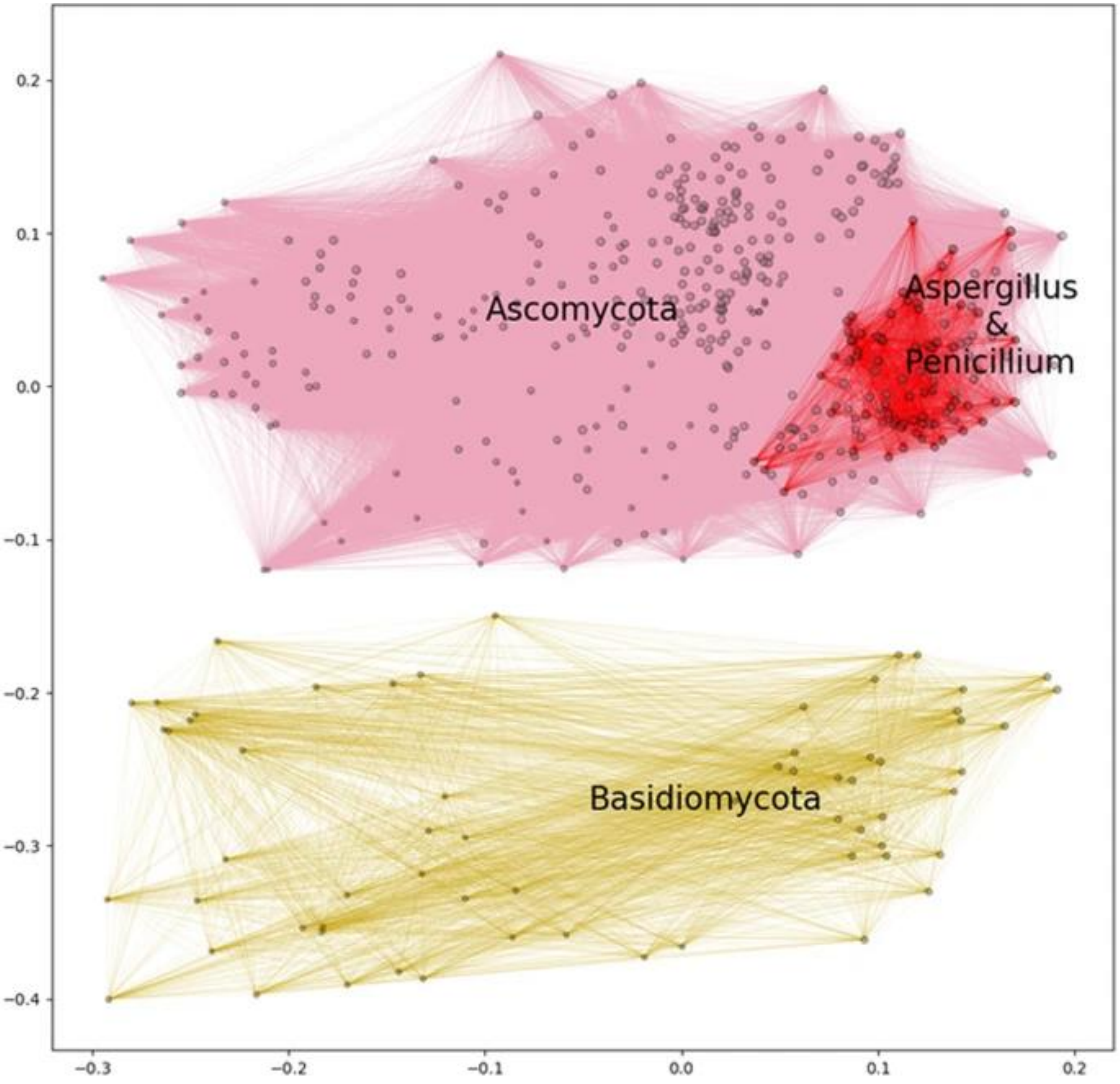
Mycena citricolor **on coffee plants, leaves and fruits, illustrates massive degradation of plant tissue, creating holes in the leaves and sunking in parts of the berries**

Photo, courtecy of Andrew Dominick, University of Wisconsin-La Crosse

Refs: CUPP Functional Annotation-based Methods. Try it!

- **CUPP**: **Barrett, K.; Lange, L.:** Peptide-Based Functional Annotation of Carbohydrate-Active Enzymes by Conserved Unique Peptide Patterns (CUPP). *Biotechnol Biofuels* **2019**, *12*, 102, doi:10.1186/s13068-019-1436-5.
- **CUPP WEB-TOOL**: **Barrett, K**.; Hunt, C.J.; **Lange, L.; Meyer, A.S.:** Conserved Unique Peptide Patterns (CUPP) Online Platform: Peptide-Based Functional Annotation of Carbohydrate Active Enzymes. *Nucleic Acids Research* **2020**, *48*, W110–W115, doi:10.1093/nar/gkaa375.
- **Function;Family (F;F) annotation and Enzyme Profile Relatedness, EPR**: **Barrett, K.**; Jensen, K.; Meyer, A.S.; Frisvad, J.C.; **Lange, L.:** Fungal Secretome Profile Categorization of CAZymes by Function and Family Corresponds to Fungal Phylogeny and Taxonomy: Example Aspergillus and Penicillium. Sci Rep 2020, 10, 5158, doi:10.1038/s4598-020-61907-1
- **HOTSPOT and F;F**: **Lange, L.; Barrett, K.; Meyer, A.S.** New Method for Identifying Fungal Kingdom Enzyme Hotspots from Genome Sequences. JoF 2021, 7, 207, doi:10.3390/jof7030207
- **+1000 JGI GENOMES**: **Kristian Barrett**, Cameron J Hunt, **Lene Lange**, Igor V Grigoriev, **Anne S Meyer,** Conserved unique peptide patterns (CUPP) online platform 2.0: implementation of +1000 JGI fungal genomes, Nucleic Acids Research, Volume 51, Issue W1, 5 July 2023, Pages W108–W114, https://doi.org/10.1093/nar/gkad385

EPR-based MDS gave a close match to Phylogeny for Asco- & Basidiomycota



Key Question:

How would EPR Enzyme profile relatedness, cluster the Basal Fungi?

Future perspectives for use of CUPP

- Optimize CUPP for function prediction of Microbiome interaction secretome
- Elucidate evolution of fungal early lineages – understanding basis for evolution of Fungi
- Develop functional prediction for Proteases and Lipases directly from genome sequence
- Learn from Nature´s hotspots of CAZymes: Improved blends for producing prebiotics from biomass
- Implement peptide based functional annotation by CUPP and include EPR, Hotspot and Blend analysis on many more types of fungi
  - Also on your favorite fungi? Let me know!

Thanks a lot for the opportunity to present -and for your kind attention

Lene

Sense of Urgency:
Climate change is already now posing a threat to stable yields! Food security is at risk in East Africa!

African counter-parts in Circular Biobased Economy & Biosolutions!

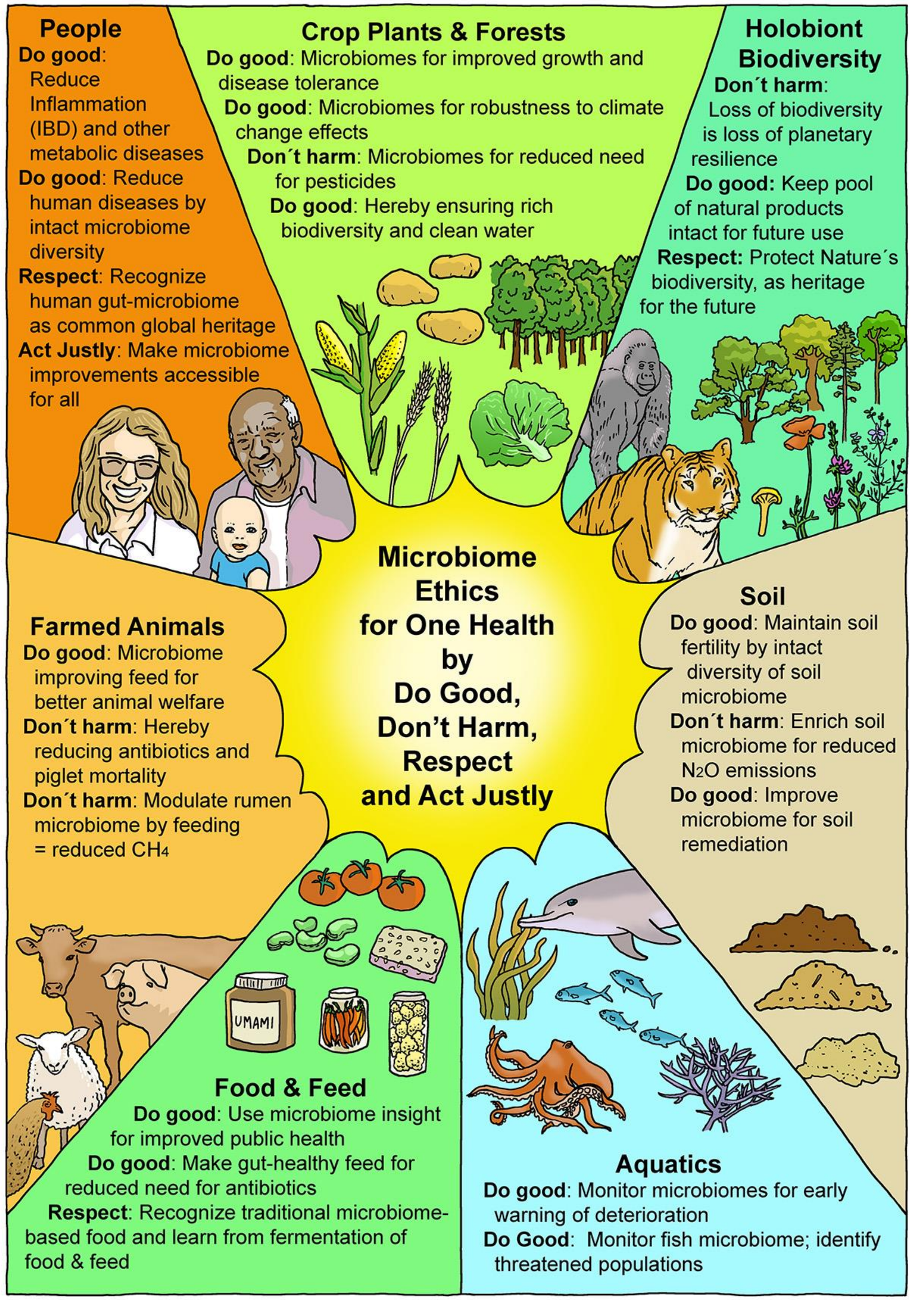
- ”BioInnovate” (East Africa, 6 countries, SIDA)
- Ghana, KNUST (West Africa; DTU, Danida funding)

New Opportunity:

A ”Circular Biobased & Biosolutions” Partnership with Africa

Building an ”African/Nordic/Danish Biosolutions Alliance” for circular&biobased, Biosolutions! -ensuring improved food security, healthy nutrition, jobs & livelihood!

Status: Malnutrition and Hunger can be the reality at the same time as industrial food processing waste/residues are piling up, rottening and polluting the environment



Microbiome Ethics for One Health by Do Good, Don't Harm, Respect and Act Justly

People

Do good: Reduce Inflammation (IBD) and other metabolic diseases

Do good: Reduce human diseases by intact microbiome diversity

Respect: Recognize human gut-microbiome as common global heritage

Act Justly: Make microbiome improvements accessible for all

Crop Plants & Forests

Do good: Microbiomes for improved growth and disease tolerance

Do good: Microbiomes for robustness to climate change effects

Don´t harm: Microbiomes for reduced need for pesticides

Do good: Hereby ensuring rich biodiversity and clean water

Holobiont Biodiversity

Don´t harm: Loss of biodiversity is loss of planetary resilience

Do good: Keep pool of natural products intact for future use

Respect: Protect Nature´s biodiversity, as heritage for the future

Farmed Animals

Do good: Microbiome improving feed for better animal welfare

Don´t harm: Hereby reducing antibiotics and piglet mortality

Don´t harm: Modulate rumen microbiome by feeding = reduced CH_4

Soil

Do good: Maintain soil fertility by intact diversity of soil microbiome

Don´t harm: Enrich soil microbiome for reduced N_2O emissions

Do good: Improve microbiome for soil remediation

Food & Feed

Do good: Use microbiome insight for improved public health

Do good: Make gut-healthy feed for reduced need for antibiotics

Respect: Recognize traditional microbiome-based food and learn from fermentation of food & feed

Aquatics

Do good: Monitor microbiomes for early warning of deterioration

Do Good: Monitor fish microbiome; identify threatened populations

- **Career: Director of Research in both Academia and Industry.** PhD and postdoc at KU. Start of career **Danida**, seed-borne plant diseases, (fungi and viruses), collaborating eight years with global south. Next: 20 years in large biotech industry (**Novo, Novo Nordisk & Novozymes**). Back to academia: Full professor and research leader in Mycology, Biotechnology & Bioeconomy at three Danish universities, **KU, AAU** and **lastly DTU**.
- **Publications**: In all >310 peer reviewed papers, books, and monographs and several patents. In all > 6450 citations, of these >3.927 after 2018. H-index 36. (Google Scholar).
- **Own company since 2018, LL-BioEconomy**. Focus: Contributing to research, technology and innovation for commercial use of enzymes for upgrading the huge amounts of biological resources now wasted. Activities: SME partner to Danish, Nordic, European & International research projects; and member of Scientific Committee, advisory to EU Commission on “Circular Biobased Europe”; and of the National Bioeconomy Panel, advisory to Danish Government.
- *For more information:* www.ll-bioeconomy.world