

Phylogenomics and Structural Diversity of Expansin-Like Proteins Associated with the Modification of Plant Cell Walls

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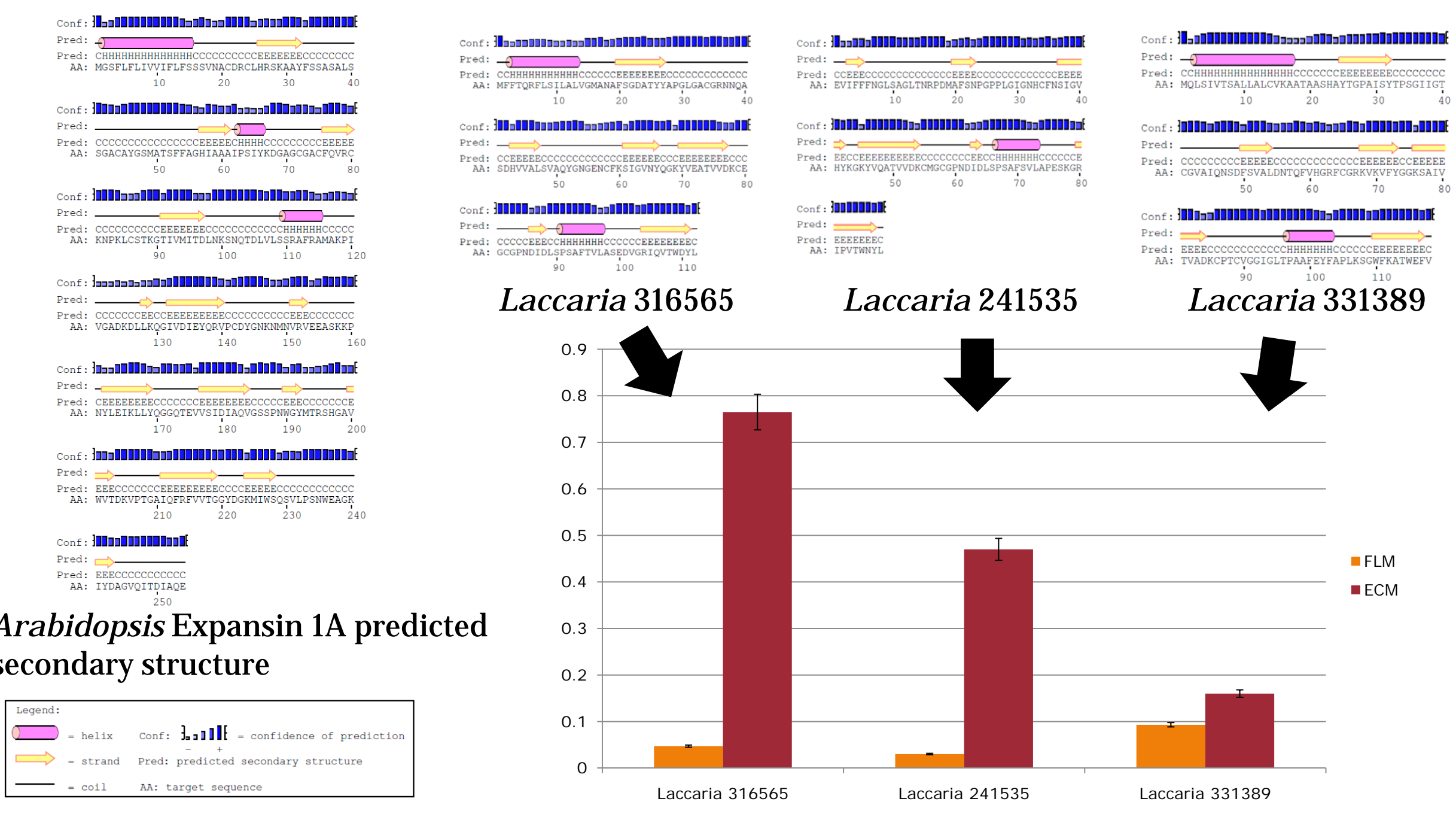
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Abstract

Expansins were first recognized as plant cell wall associated proteins responsible for the loosening of cell walls during plant growth. Expansins have subsequently been related to numerous processes involving plant cell wall modification, including pollen tube growth within the flower stigma and acid-growth of plant cells. Although first recognized in plants, the presence of a homologous protein found outside of the plant lineage was first described in the cellulose degrading Ascomycete fungus *Trichoderma reesei*. These proteins are also noted for their role in allergic reactions to both plant pollen and fungal spores. Plants with sequenced genomes have, on average, more than 40 copies of expansin and expansin-like proteins. With upwards of 50 fungal genomes and more than 6400 bacterial genomes sequenced, a large number of these proteins have been identified in other lineages. These proteins show approximately 40% sequence homology when measured across all taxonomic lineages. Both bacterial and fungal proteins share homology to plant expansins, and appear to have evolved as a mechanism for interacting and modifying both living and deceased plant cell walls. Here we present a large-scale multi-kingdom phylogeny of the expansin protein family, including proteins with homology to Barwin-like endoglucanases and membrane-bound lytic murein transglycosylases (MLTAs). Plant expansins can be designated into four classes which show limited homology to both fungal and bacterial expansins. Transcription of expansin-like proteins has been recently shown to be highly upregulated in mycorrhizal fungi, notably *Laccaria bicolor* and *Glomus intraradices*, during the colonization of the host plant roots. Phylogenetic analysis shows clear Basidiomycete and Ascomycete lineages for three clades of fungal expansins. *Laccaria bicolor*, for example, presumably has 11 expansins, which have diversified via both tandem and segmental genome duplications. Using the model mycorrhizal association of *Laccaria bicolor* and *Populus trichocarpa*, we elucidate expansin gene expression during the formation of mycorrhizal roots.

Figure 1 - Homology of putative Expansins from the Basidiomycete *Laccaria bicolor* (S238N) to *Arabidopsis* Expansin 1A and expression of three expansins of *Laccaria* in free living mycelium (FLM) and ectomycorrhizal root tips with Hybrid *Populus*.



Introduction

- Expansins are involved in plant cell wall-loosening, but the exact mechanism for this is not completely understood.
- Originally found in plants, Expasin-like proteins are found in the fungi as well as bacterial precursors.
- The activity of plant expansins, as well as expansins of fungus *Laccaria bicolor*, have been localized using immunochemical identification and electron microscopy.
- Studies using transgenic plants suggest a broad range of biological roles of expansins in diverse aspects of plant growth and development, such as cell wall extension, fruit softening, abscission, floral organ development, symbiosis, and the response to environmental stresses.

Results & Discussion

- PHYLOGENETIC ANALYSIS:** A database was constructed from data derived from public resources and in-house sequencing projects. Phylogenetic analysis (Figure 2 & 4) was conducted on eukaryotic Expansin proteins from Plants, Fungi, and fungal-like organisms. Sequences were aligned using Kalign (Lassman & Sonnhammer 2005) and then manually edited for accuracy.
- EXPRESSION & HOMOLOGY OF *LACCARIA* EXPANSINS:** The expression of three *Laccaria* expansins (Figure 1) were measured in free living mycelium and in ectomycorrhizal roots. Secondary structure homology is compared with *Arabidopsis* Expansin A1.
- EXPANSIN HOMOLOGY:** The homology of expansin proteins across Plants and Fungi is shown in Figure 3. Numerous conserved regions exist for all expansins.

Figure 2 – Phylogeny of representative Expansins from Plants (Monocots & Dicots) and Fungi (Ascomycetes & Basidiomycetes). Homology of species shown here is located in Figure 3. The evolution from bacterial expansins in under investigation.

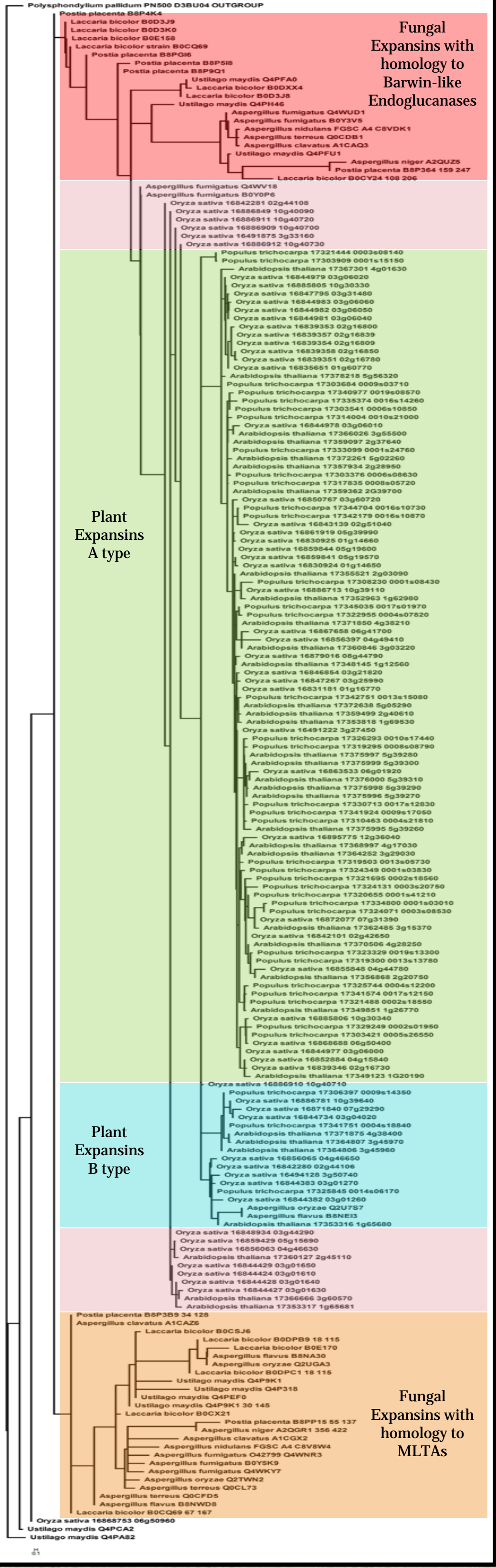


Figure 3 - Homology of putative Expansins from Plant lineages (Monocots & Dicots) and Fungal lineages (Basidiomycete & Ascomycetes) from key species with sequenced genomes. Alignment shows regions of homology and conservation across both plant and fungal lineages.

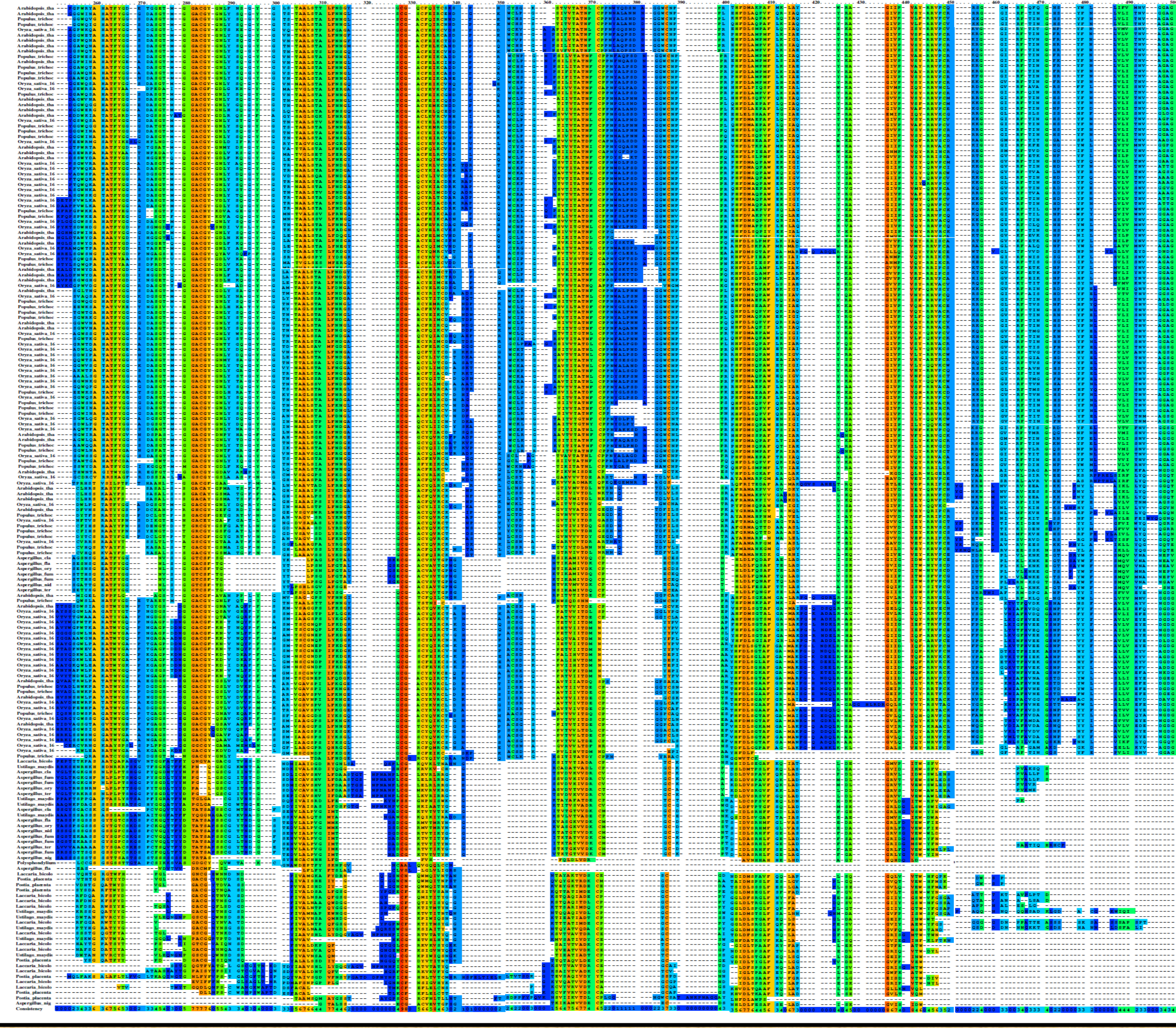
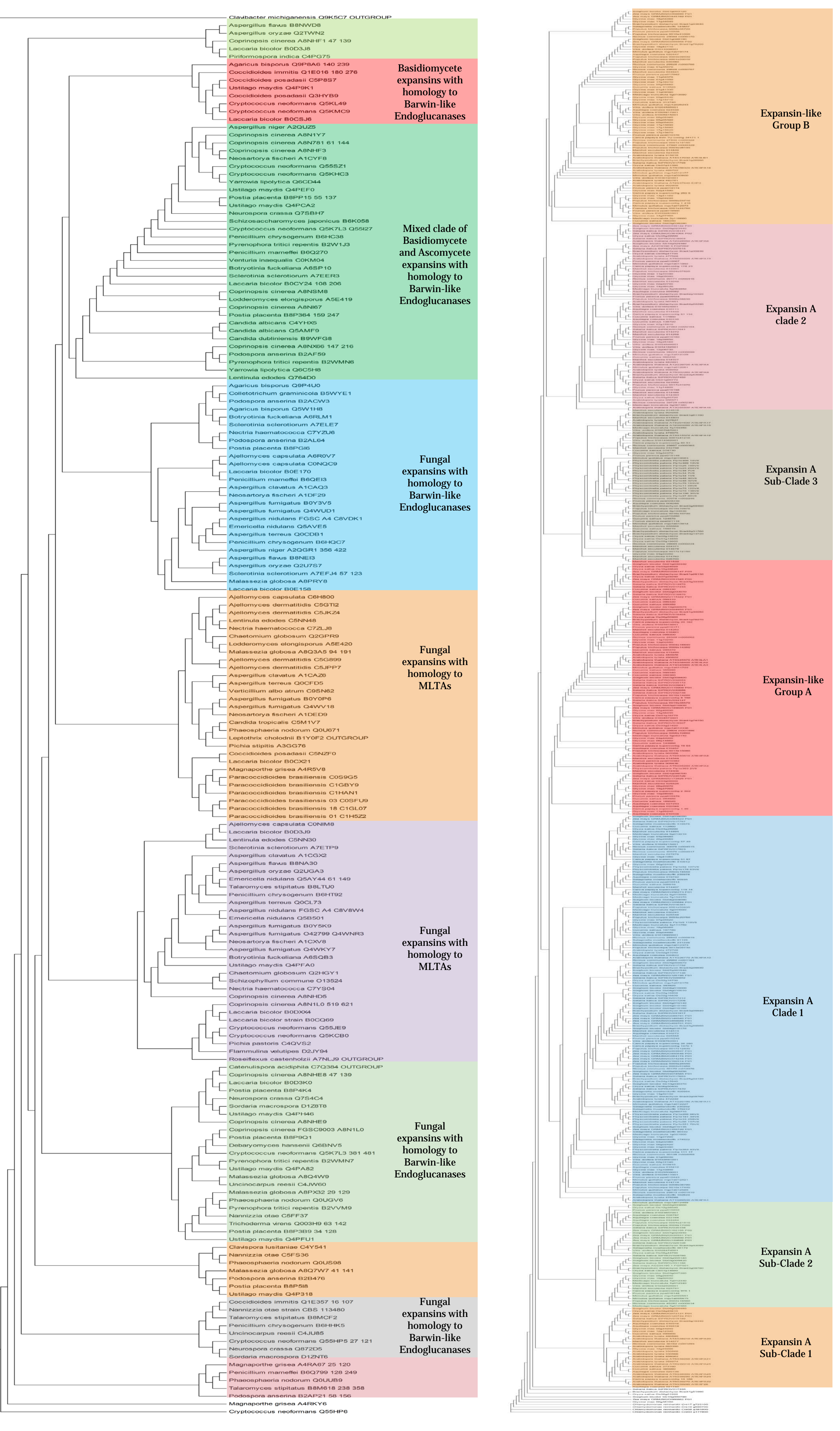


Figure 4 – Phylogeny of Expansin and Expansin-like proteins constructed public databases and from highly significant BLAST hits (less than 2e-20) across plant and fungal EST libraries. Phylogeny constructed in PAUP* using the Maximum Parsimony algorithm. Similar trees were obtained using the Mr. Bayes and PhyML computer programs. Tree on the right represents 578 plant accessions and the tree on the left represents 198 fungal accessions.



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