

ENZYME DISCOVERY AND SPECIFICITY FINGERPRINTS BY ANALYSIS OF CORRELATED POSITIONS IN CAZY FAMILY GH65

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While the continuously growing amount of protein sequences in databases confers an enormous potential, it is also difficult to harness. Some enzyme families have grown so big that it becomes increasingly challenging to distinguish between the different enzyme specificities or to uncover new functionalities within one enzyme family. In this study, we conducted an in-silico analysis in the CAZy Glycoside Hydrolase Family 65 (GH65) to help navigate its sequence diversity. By studying correlated mutations, we revealed specificity-determining positions that facilitate the annotation of the family's phylogenetic tree based on distinct sequence patterns (Figure 1a). Furthermore, by searching these positions for amino acid motifs that do not match those found in previously characterized enzymes from GH65, several clades that may harbor new functions could be identified. To validate this hypothesis, three enzymes from across these clades were expressed in *E. coli* and their substrate profile was mapped. One of those enzymes, originating from the bacterium *Mucilaginibacter mallensis*, possessed a previously unreported activity: the hydrolysis of kojibiose (α -1,2-glucobiose) and α -1,2-oligoglucans with high specificity (Figure 1b). This enzyme was the first characterized representative of a novel enzyme specificity, which received the name *kojibiose hydrolase* (EC 3.2.1.216). This work illustrates a convenient and generic strategy for mapping the natural diversity of enzyme families and smartly mining the ever-growing number of available sequences in the quest for novel specificities [1].

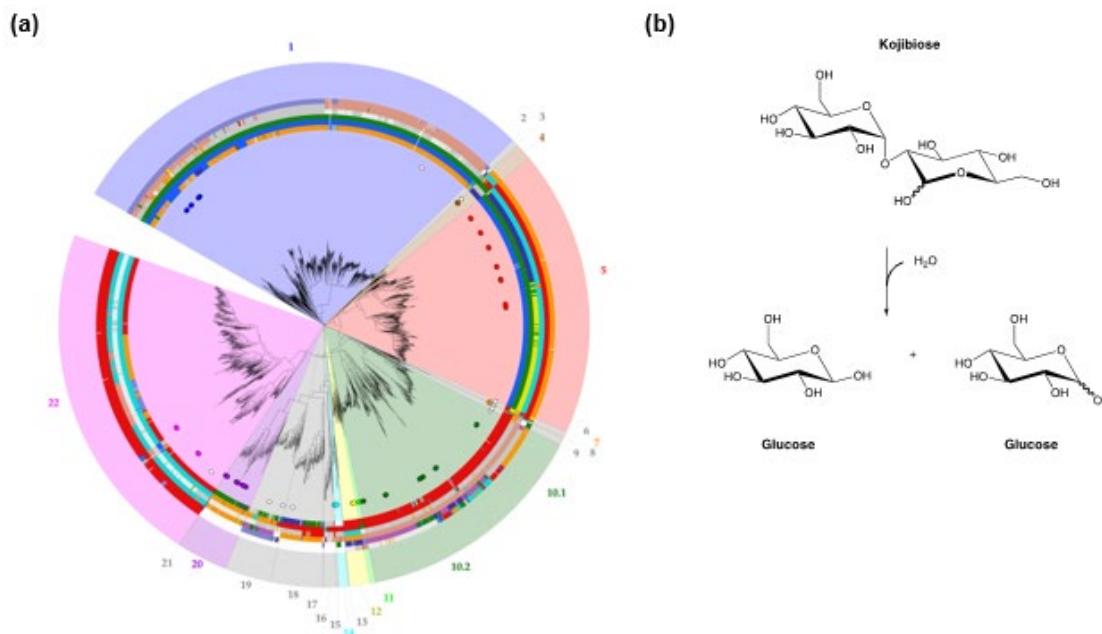


Figure 1 – (a) Annotated phylogenetic tree of CAZy family GH65, with specificity-determining positions visualized as colored rings around the tree. Details available in [1]. (b) Reaction catalyzed by kojibiose hydrolase: hydrolysis of the α -1,2 glucosidic bond of kojibiose results in the release of two glucose molecules.