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Starch is the second most abundant plant-derived biomass and a major feedstock in non-food industrial applications and first generation biofuel production. In contrast to lignocellulose, detailed insight into fungal degradation of starch is currently lacking. This study explores the secretomes of Aspergillus nidulans grown on cereal starches from wheat and high-amylose (HA) maize, as well as legume starch from pea for 5 days. Aspergillus nidulans grew efficiently on cereal starches, whereas growth on pea starch was poor. The secretomes at days 3-5 were starch-type dependent as also reflected by amylolytic activity measurements. Nearly half of the 312 proteins in the secretomes were carbohydrate-active enzymes (CAZymes), mostly glycoside hydrolases (GHs) and oxidative auxiliary activities (AAs). The abundance of the GH13 α-amylase (AmyB) decreased with time, as opposed to other starch-degrading enzymes, e.g., the GH13 AmyF, GH15 glucoamylases (GlaA and GlaB), and the GH31 α-glucosidase (AgdE). Two AA13 LPMOs displayed similar secretion patterns as amylolytic hydrolases and were among the most abundant CAZymes. The starch-active AnLPMO13A that possesses a CBM20 carbohydrate-binding module dominated the starch-binding secretome fraction. A striking observation is the co-secretion of several redox-active enzymes with the starch-active AA13 LPMOs and GHs, some at high abundance. Notably nine AA9 LPMOs, six AA3 sub-family 2 (AA_2) oxidoreductases, and ten AA7 glycosidosaccharide oxidases were identified in the secretomes in addition to other non-CAZyme oxidoreductases. The co-secretion and high abundance of AA13 LPMOs are indicative of a key role in starch granule deconstruction. The increase in AA13 LPMO abundance with culture time may reflect accumulation of a more resistant starch fraction towards the later stages of the culture. The identification of AmyR sites upstream AA13 LPMOs unveils co-regulation of LPMOs featuring in starch utilization. Differential deployment of amylolytic hydrolases and LPMOs over time suggests additional regulatory mechanisms. The abundant co-secretion of distinct AA3 and AA7 oxidoreductases merits further studies into their roles and possible interplay with LPMOs and other enzymes in the deconstruction of starchy substrates. The study reports for the first time the biological significance of LPMOs in starch degradation and the temporal interplay between these and amylolytic hydrolases.

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