An extracellular cell-attached pullulanase confers branched α-glucan utilization in human gut Lactobacillus acidophilus

Of the few predicted extracellular glycan-active enzymes, glycoside hydrolase family 13 subfamily 14 (GH13_14) pullulanases are the most common in human gut lactobacilli. These enzymes share a unique modular organization, not observed in other bacteria, featuring a catalytic module, two starch binding modules, a domain of unknown function, and a C-terminal surface layer association protein (SLAP) domain. Here we explore the specificity of a representative of this group of pullulanases, LaPul13_14 and its role in branched α-glucans metabolism in the well characterized Lactobacillus acidophilus NCFM that is widely used as a probiotic. Growth experiments of L. acidophilus NCFM on starch-derived branched substrates revealed preference for α-glucans with short branches of about two to three glucosyl moieties over amylopectin with longer branches. Cell-attached debranching activity was measurable in the presence of α-glucans but was repressed by glucose. The debranching activity is conferred exclusively by LaPul13_14 and is abolished in a mutant strain lacking a functional LaPul13_14 gene. Hydrolisis kinetics of recombinant LaPul13_14 confirmed the preference for short branched α-glucan oligomers consistent with the growth data. Curiously, this enzyme displayed the highest catalytic efficiency and the lowest Km reported for a pullulanase. Inhibition kinetics revealed mixed inhibition by β-cyclodextrin suggesting the presence of additional glucan binding sites besides the active site of the enzyme, which may contribute to the unprecedented substrate affinity. The enzyme also displays high thermostability and higher activity in the acidic pH range reflecting adaptation to the physiologically challenging conditions in the human gut.IMPORTANCE Starch is one of the most abundant glycans in human diet. Branched α-1,6-glucans in dietary starch and glycogen are non-degradable by human enzymes and constitute a metabolic resource for the gut microbiota. The role of health-beneficial lactobacilli prevalent in the human small intestine in starch metabolism remains unexplored in contrast to colonic bacterial residents. This study highlights the pivotal role of debranching enzymes in the break-down of starchy branched α-glucan oligomers (α-limit dextrins) by human gut lactobacilli exemplified by Lactobacillus acidophilus NCFM, which is one of the best characterized strains used as probiotics. Our data bring novel insight into the metabolic preference of L. acidophilus for α-glucans with short α-1,6-branches. The unprecedented affinity of the debranching enzyme that confers growth on these substrates reflects its adaptation to the nutrient-competitive gut ecological niche and constitutes a potential advantage in cross-feeding from human and bacterial dietary starch metabolism.