FUNCTIONAL METAGENOMIC SCREENING APPROACH FOR DISCOVERY OF NEW GLYOSIDE PHOSPHORYLASES

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Glycoside phosphorylases (GPs) have recently been recognized as potentially useful biocatalysts for the synthesis and biotransformation of glycans. These enzymes ordinarily carry out phosphorolysis of the glycosidic linkage by transferring a glycosyl moiety from the non-reducing end of a di- or polysaccharide substrate onto inorganic phosphate, thereby cleaving the glycosidic bond and generating a sugar-1-phosphate. GPs distinguish themselves from most carbohydrate-active enzymes in that the hydrolytic free energy associated with the ester-linkage of the sugar-1-phosphate product is roughly equivalent to that of the glycosidic linkage in the glycan substrate. Therefore, the equilibrium position can be tipped in favour of glycoside synthesis by manipulation of reaction conditions. GPs thus have considerable potential for the assembly of glycans, especially since their reversibility would allow the use of one GP to degrade an inexpensive glycan to produce a pool of sugar-1-phosphates, while a second GP could be deployed to use those sugar-1 phosphates as donors to synthesize a different, more valuable target glycan. The bottleneck in this approach, however, is the limited range of GPs available, which restricts the classes of glycan that can be assembled. To help increase the spectrum of known GPs available, we have turned to metagenomics as a means to discover new enzymes belonging to this class. We have adapted the molybdenum blue reaction to a high-throughput plate-based metagenomic screen for the discovery of GPs. Our method utilizes the reverse phosphorolysis ability of GPs by coupling inorganic phosphate released during glycan synthesis to the molybdenum blue reaction. Therefore, GP activity can be identified by incubating metagenomic clones with appropriate donor sugar-1-phosphates and acceptor glycans, then monitoring inorganic phosphate accumulation by measuring formation of molybdenum blue. Our pilot screen was optimized to identify cellulose degrading GPs and yielded 7 novel GP ORFs, all from CAZy family GH94. To our knowledge this is the first high-throughput functional metagenomic screen for GP activity. Looking ahead, we have planned to further adapt the screening method so it may identify GP activity from other CAZy families, beyond GH94. The activities that can be identified are dependent on the combination of donor and acceptor substrates used. By mixing and matching different substrates we will be able to narrow or broaden the scope of activities that can be detected within a single screen.