DISCOVERY OF NEW BACTERIAL A2,3- AND A2,6-SIALYLTRANSFERASES WITHOUT UNDESIRED HYDROLYTIC ACTIVITIES FOR EFFICIENT GLYCAN SYNTHESIS

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The development of efficient enzymatic tools for sialylated glycan synthesis remains a critical challenge in glycobiotechnology. This study addresses longstanding limitations in sialyltransferase (SiaT) applications by identifying novel bacterial enzymes with superior catalytic properties. While bacterial SiaTs are generally preferred over mammalian orthologues due to simpler recombinant production and broader substrate specificity [1,2], their utility has been constrained by undesirable hydrolytic side activities toward CMP-Neu5Ac donors and sialylated products [3,4]. Previous engineering attempts to suppress these activities resulted in compromised catalytic efficiency or thermal stability [5]. Here, we report the discovery of previously uncharacterized bacterial α2,3- and α2,6-SiaTs that maintain native transferase activity while exhibiting negligible hydrolase, sialidase, and trans-sialidase activities. The absence of hydrolytic side reactions enables unprecedented reaction yields (>95%) in gram-scale syntheses of complex glycans, including sialylated human milk oligosaccharides. Here, we demonstrate the synthesis of sialyllactoses. This breakthrough establishes a new generation of biocatalysts for industrial glycoconjugate production while providing insights into the molecular determinants of sialyltransferase promiscuity.

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