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Enzymatic treatment of biomass is an environmentally friendly method to obtain a range of value-added products, such as biofuels, animal feed or food ingredients. The objective of this PhD study was to biocatalytically produce biofunctional food ingredients – human milk oligosaccharides decorated with sialic acid from casein glycomacropeptide obtained from dairy side streams. In addition, the biocatalysts employed in this study, i.e., a sialyltransferase and a sialidase, were subjected to protein engineering to alter the enzyme’s regioselectivity and to improve hydrolase activity, respectively. A recombinant Pasteurella multocida sialyltransferase (EC 2.4.99.-), namely PmST, exhibiting promiscuous trans-sialidase activities was examined. The enzyme catalysed α-2,3- and α-2,6-sialylation of lactose using either 2-O-(p-nitrophenyl)-α-D-N-acetylneuraminic acid or casein glycomacropeptide as a sialyl donor. This is the first study reporting α-2,6-trans-sialidase activity of this enzyme. Using response surface design allowed identification of two differently optimised conditions for PmST-catalysed production of 3'-sialyllactose and 6'-sialyllactose, giving maximum yields of 2.8 mM and 3.3 mM from casein glycomacropeptide (9 mM bound sialic acid), respectively. The k cat / K m value for PmST catalysing 6'-sialyllactose synthesis using 3'-sialyllactose as donor was 23.2 ± 0.7 M⁻¹ s⁻¹. Further, the enzyme was capable of catalysing synthesis of both 3'- and 6'-sialylated galactooligosaccharides with use of galactooligosaccharides as acceptors. Secondly, we examined the regioselectivity of five designed mutants of PmST catalysing synthesis of 3'- and 6'-sialyllactoses using casein glycomacropeptide and lactose as substrates. The mutants PmST E271F, PmST R313Y and PmST E271F/R313Y preferentially catalysed synthesis of 3'-sialyllactose over 6'-sialyllactose. The best mutant PmST E271F/R313Y for α-2,3-trans-sialylation gave a maximum 3'-sialyllactose yield of 4.5 mM from casein glycomacropeptide (9 mM bound sialic acid). Another mutant PmST P34H displayed a distinct preference for 6'-sialyllactose synthesis throughout the reaction, though the total sialyllactose yield was consistently and significantly lower than that using the wild type enzyme. PmST P34H had a 980-fold increase in α-2,6-sialyltransferase activity compared to the wild type enzyme, while its α-2,3-sialyltransferase activity was almost abolished. The k cat / K m value for PmST P34H catalysing 6'-sialyllactose synthesis using 3'-sialyllactose as donor was 31.2 M⁻¹ s⁻¹. Moreover, both the wild type enzyme and PmST P34H were capable of catalysing the hydrolysis and transfer of α-2,6 bound sialic acid.

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