

7. Summary

In recent years, an increasing effort has been made to screen for functional food components. In this context the definition of the term “functional” describes components displaying health beneficial effects. Oligosaccharides are of particular interest due to their prebiotic attributes, responsible for a proper intestinal flora and thus stabilizing effects of the immune system. Synthetic access for these oligosaccharides on larger scale of is of particular interest to the food industry.

In this work the selectivity and the donor specificity of the α -hydrolases RAF-A and aGaB as well as the β -hydrolase BgLT were studied. Novel donors have been synthesised, and enzymatic approaches for the synthesis of new oligosaccharides were developed. Further, upscaling with β -galactosidases from bovine testes, *Bacillus circulans* and *Aspergillus oryzae* were accomplished successfully.

RAF-A is an α -galactosidas with distinctive $\alpha(1\rightarrow6)$ specificity for monomeric acceptors such as methyl glucoside **14**, methyl gactoside **15** or methyl mannoside **16**. In case of methyl glucoside **14** the $\alpha(1\rightarrow4)$ linked derivative was found as side product. Further, it was possible to diversify the position of galactosylation employing selectively 6-*O*-protected acceptors. Using disaccharide acceptors such as sucrose and isomaltulose only the $\alpha(1\rightarrow6)$ regioisomeric derivatives were obtained. RAF-A showed other interesting donor specificities because in addition to *p*NP- α -D-galactopyranoside (**19**), even mellibiose **27** and α -D-galactopyranosyl fluoride (**18**), as well as partially α -D-fucopyranosyl fluoride (**21**) and β -L-arabinopyranosyl fluoride (**24**) were accepted as a donor substrate.

aGaB is a thermophilic α -hydrolase with a temperature optimum at 65 °C, showing an exclusive $\alpha(1\rightarrow6)$ specificity. This enzyme showed a significant selectivity and only accepted *p*NP- α -D-galactopyranoside (**19**), mellibiose **27** und α -D-galactopyranosyl fluoride (**18**).

BgLT is a thermophilic β -galactosidase, which turned out to establish novel $\beta(1\rightarrow3)$ linkages to the applied acceptors and thus may be considered a distinguished substitute for the β -galactosidase from bovine testes. In addition to lactose **28**, *p*NP β -D-galactopyranoside (**85**) and pyridinyl β -D-galactopyranoside (**20**) BgLT accepted even pyridinyl β -D-fucopyranoside (**23**) and pyridinyl α -L-arabinopyranoside (**26**) as donor substrates and formed $\beta(1\rightarrow3)$ bonds exclusively. In the range of acceptor substrates sucrose (**11**), isomalt (**12**), isomaltulose (**13**) and methyl α -D-glucopyranoside (**14**) also allyl- α -*N*-acetylglucosaminide **17**, could be galactosylated

successfully with this may open an interesting synthetic passway to new chitooligomers.

Further it was shown, that transglycosylation succeeded well with both the β -galactosidases from bovine testes and *Aspergillus oryzae* without a specific buffer only in pH adjusted aqueous media. Finally these enzymes, as well as the β -galactosidase from *Bacillus circulans* could be employed to attain β -(1 \rightarrow 3)-, β -(1 \rightarrow 4) and β -(1 \rightarrow 6)-galactosylated structures on a gram scale.

The present work shows novel pathways for the enzymatic synthesis of oligosaccharides and emphasises the versatile applications of hydrolases with natural and artificial donors in organic synthesis.