

# Development of a fully enzymatic conversion process from marine chitin to chitosan oligomers

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# Summary

Polysaccharides such as cellulose and chitin belong to the most abundant biopolymers in the world and take over essential roles in the structural conformations in plant- and animal kingdoms. Both polymers are also widely integrated in chemical industries and secondary products are already fully integrated in our daily lives. Recent structural and functional descriptions of chitin-based degradation products such as chitosan and chitosan oligomers revealed an impressive variety of intrinsic properties that are however highly sensitive to changes of physicochemical characteristics. As the predominant harsh chemical degradation processes of chitin lack the required reaction specificity and selectivity, an efficient production process for defined chitosan products still remains a major challenge. Enzymes are natural functional macromolecules and key factors in biocatalytic processes typically employed for a more specific and selective conversion of molecules. As an alternative to chemical conversions, enzymatic processes can furthermore foster a reduction of environmental impact and elimination of toxic production residues.

The objective of this thesis is to integrate chitinolytic enzymes for the controlled degradation of chitin to defined chitosan oligomers, which can be used for fiber functionalization in medical applications. In particular, novel chitinases and chitin-deacetylases originating from a novel marine bacterial strain will be implemented in *in vitro* enzyme cocktails for the controlled depolymerization and deacetylation of marine chitin. Furthermore, the hydrolytic potential of enzyme cocktails will be assessed to alter physicochemical properties of chitosan oligomers, thus changing intrinsic physiological effects.

An overview on the currently established methods for the preparation of defined chitosan oligomers is given in **Chapter 2**. Chemical methods and their optimization approaches for depolymerization and deacetylation of chitin are summarized and limitations are highlighted. The role and function of chitinolytic enzymes by means of chitinases and chitin-deacetylases and state-of-the-art of enzymatic degradation

processes are stressed. Statistical methodologies and biotechnological approaches to improve the relevance of fully enzymatic approaches are discussed.

In order to establish a fully enzymatic production process for chitosan oligomers, effective chitinolytic enzymes are required to carry out depolymerization and deacetylation reactions. A polyphasic characterization and phylogenetic classification of a novel chitinolytic bacterium (Chi5) are described in **Chapter 3**. Experimental evidence is given confirming the capability of the bacterium to hydrolyze marine chitin using mixtures of chitinolytic enzymes.

Five different genes each for chitinases and chitin-deacetylases were identified after a whole-genome sequencing of Chi5 and recombinant techniques were used to express the genes by *Escherichia coli* BL21. The enzymes are further explored and characterized in **Chapter 4** and **Chapter 5** to assess reaction optima, enzyme kinetics, substrate specificities and product characteristics. It is revealed that the enzymes are active on soluble as well as insoluble substrates and are capable to degrade chitin to oligomers of a low degree of polymerization and carry out a partial deacetylation.

A holistic fully enzymatic approach for the generation of chitosan oligomers is explored in **Chapter 6**. The design-of-experiments approach is used to model optimum enzyme combinations for depolymerization and sequential deacetylation reactions in order to increase conversion rates and to alter product properties in a controllable manner.

Overall conclusions, future prospects and further recommendations of this thesis are summarized in **Chapter 7**. The scientific and social impact of the research in this thesis is presented in **Chapter 8**.