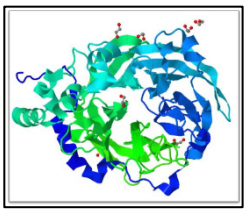

Stabilization of levansucrase, modulation of its specificity and a search for the improved synthesis of novel fructooligosaccharides and levans

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Abstract

Fructooligosaccharides (FOSs) are proven prebiotics capable of promoting the population of healthy bacteria within the colon. Levansucrase (EC 2.4.1.10, LS) using sucrose as an fructose donor, LS is capable of producing short-chained FOSs to large polysaccharides with specific β(2→6) linkages. LS is limited commercially by low thermal stability and poor product specificity. LS produced from *Bacillus amyloliquefaciens* was immobilized onto various synthetic and natural supports in order to increase thermal stability. Glyoxyl agarose-IDA-Cu offered a high retention of activity and protein yield with a stabilization factor 14 times higher than that of the native enzyme. Response surface methodology was used to investigate and optimize the immobilization parameters used. High retention of activity (71%), protein yield (55%), activity yield (45%) and stability at 50°C (4.6 stabilization factor) was obtained using the optimized immobilization conditions, along with the crosslinker polyethylenimine. Genome mining was performed in order to discover new LS enzymes with increased transfructosylating activity, decreased hydrolytic activity and increased levan forming capability. Highly active candidates, the LSs from: *Paraburkholderia graminis, Beijerinickia indica subsp. indica, Gluconobacter oxydans, Novospingobium aromaticivorans* and *Vibrio natriegens* were selected for further study of their kinetic profiles and acceptor specificities. The LS from *B. graminis* produced a large amount of FOSs (164 g/L) while *V. natriegens* catalyzed the synthesis of an abundant amount of levan (84 g/L). In the acceptor specificity study, it was shown that all the enzymes were able to utilize the alditol, sorbitol, to a varying degree while the enzymes from *V. natriegens*, *N. aromaticivorans*, *B. graminis* and *B. indica subsp. indica* were able to utilize the benzene diol, catechol as an acceptor for fructose, opening up the possibility for the production of new novel transfructosylated products.

About the Candidate

Andrea, a Montreal native, holds a B.Sc. degree in chemistry from McGill University. Since 2011, she has been working under the supervision of Professor Salwa Karboune, in the department of Food Science, on the production of novel prebiotics. She has been fortunate to work in collaboration with Dr. Cesar Mateo at the Instituto de Catalisis y Petrochemica in Spain and Professor Veronique de Berardinis at the Genoscope Centre in France.