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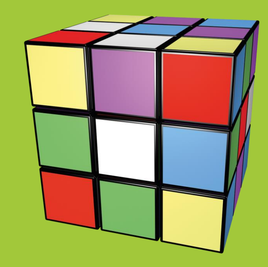

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Purification and characterisation of a β-fructofuranosidase from *E. coli.*

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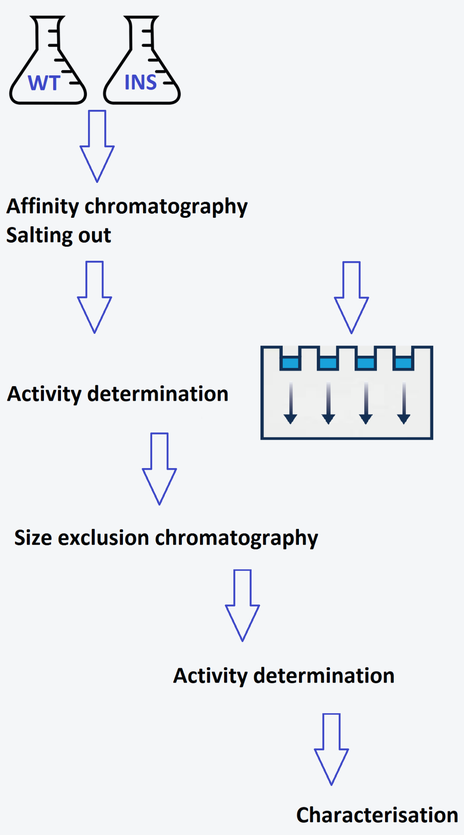
Introduction

E. coli **from intestinal cultures from baby's was seen growing on raffinose. This has never been described before. In the genome a new protein responsible for the degradation was found. It encoded a fructosidase activity that is able to cleave terminal fructose redidues from sugars. The usual way for *E. Coli* to break down sugars is via the phosphotransferase system (PTS) where sugars are phosphorylated, taken up and internally hydrolyzed by a sugarphosfatase. Several similar enzymes with fructosidase activity have already been characterised in different bacteria species (*C. glutamicum* and *B. infantis*). We expect it to have similar properties as these enzymes.**

Material and methods

The protein responsible for the activity was placed before a 6xHIS-Tag and behind a constitutive promotor. The purification was run with an empty vector (Wild Type) and a vector with insert (Insert) inserted in *E. coli.* Cell lysates were separated on a nickel affinity column and fractions were salted out. Next, the ability of the elutes to break down sucrose into fructose was measured. The elutes were also separated on gels. The most active fractions were separated by size and finally the most active fractions were selected for characterisation.

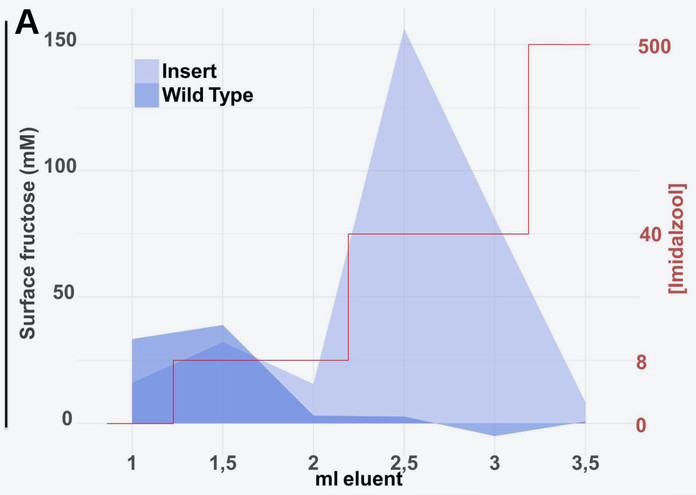


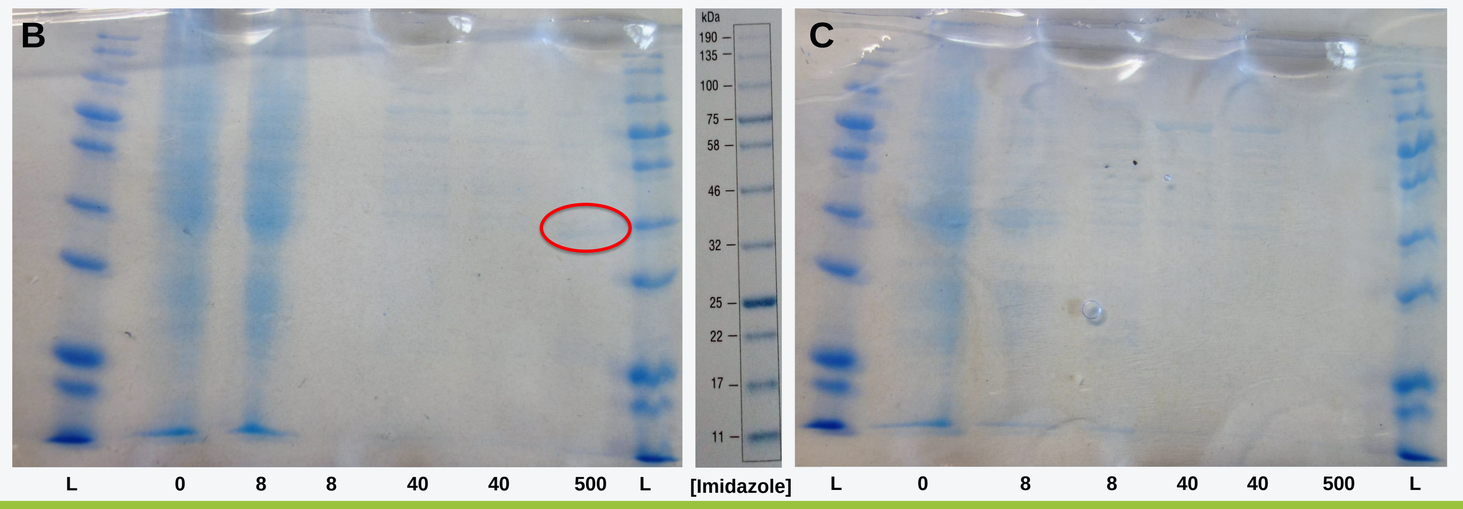
Conclusion

The fructosidase activity couldn't be directly linked to the purified 6xHis-tag protein. Characterisation of the semi-crude enzyme showed that the enzyme has a maximum activity at pH 6,6 and is able to hydrolyze sucrose and raffinose. The K_m and V_{max} values for sucrose were 10,5 mM and 4,1 µmol/min. Based on these values it is classified as a β-fructofuranosidase.

Activity is not linked to 6xHis-Tag

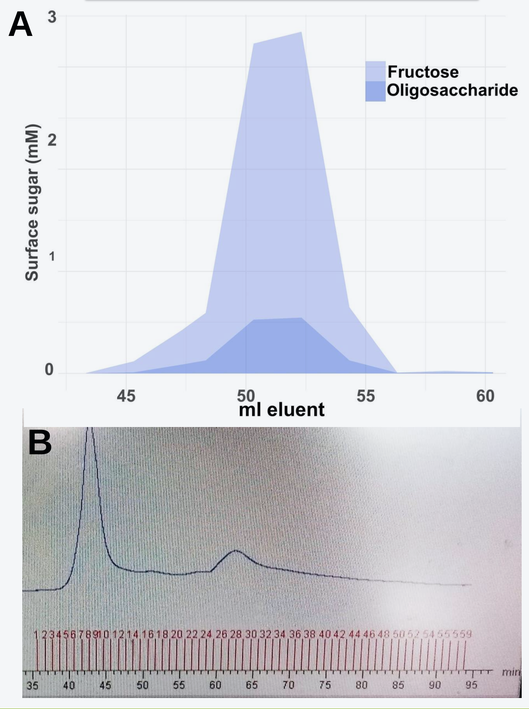
A. **After separation on a nickel affinity column most of the sucrose degrading activity is located in the fractions eluted with 40 mM imidazole.**
B. **This activity is not directly linked to the 6xHIS-Tag protein that eluted at 500mM imidazole in the *E. coli* with insert.**
C. **The *E. coli* without insert does not have an 6xHIS-Tag protein and also doesn't have high fructosidase activity.**





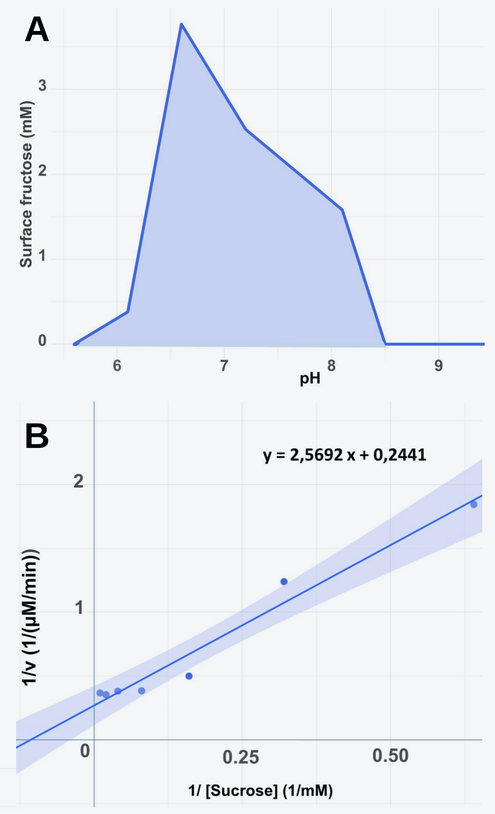
Unpure final extract

A. **After size exclusion the active fractions show formation of an oligosaccharide.**
B. **The chromatogram shows a lot of proteins for the active fractions.**



Characterisation

A. **Most sucrose degradation is seen at pH 6,6. There is fast decay in activity when the pH drops below 6,6. The decay of activity is slower in more basic pH.**
B. **The K_m is estimated at 10,5 mM and the V_{max} at 4,1 µM/min for sucrose.**



C. **The crude extract of the *E.coli* with insert is able to degrade both sucrose and raffinose efficiently.**