SUPPLEMENTARY INFORMATION

Alternative method for cloning, production and purification of recombinant protein

The gene encoding SaCsn75A, excluding the part coding for a putative 26-residue signal peptide, was amplified from *Streptomyces avermitilis* genomic DNA by polymerase chain reactions with the following primers (synthesized at Eurofins MWG Operon, Germany): *SaCsn75A*-F 5' CGAAGATCTTCCCCGTCCGGCACCAAGGC 3' and primer *SaCsn75A*-R 5' CAGAAGCTTTCAGTTGTTCTGGAGGAACTGC 3'. A BglII and a HindIII site were incorporated at the start and the end of the gene, respectively, to enable construction of an in-frame N-terminal His tag-fused construct in the pBAD/HisB(s) vector. This vector is a variant of the commercial vector pBAD/HisB (Invitrogen, USA) with a shortened region between the N-terminal polyhistidine tail and the down-stream multiple cloning site. In the final construct, the expression of the gene is under the transcriptional control of the arabinose-regulated araBAD promoter. The gene product consists of the sequence MAHHHHHHHRS followed by the mature SaCsn75A. The resulting plasmids were transformed into *E. coli* TOP 10 cells (Invitrogen). DNA sequencing was performed using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Perkin Elmer / Applied Biosystems, Foster City, CA, USA) and an ABI PRISM® 3100 Genetic Analyser (Perkin Elmer / Applied Biosystems).

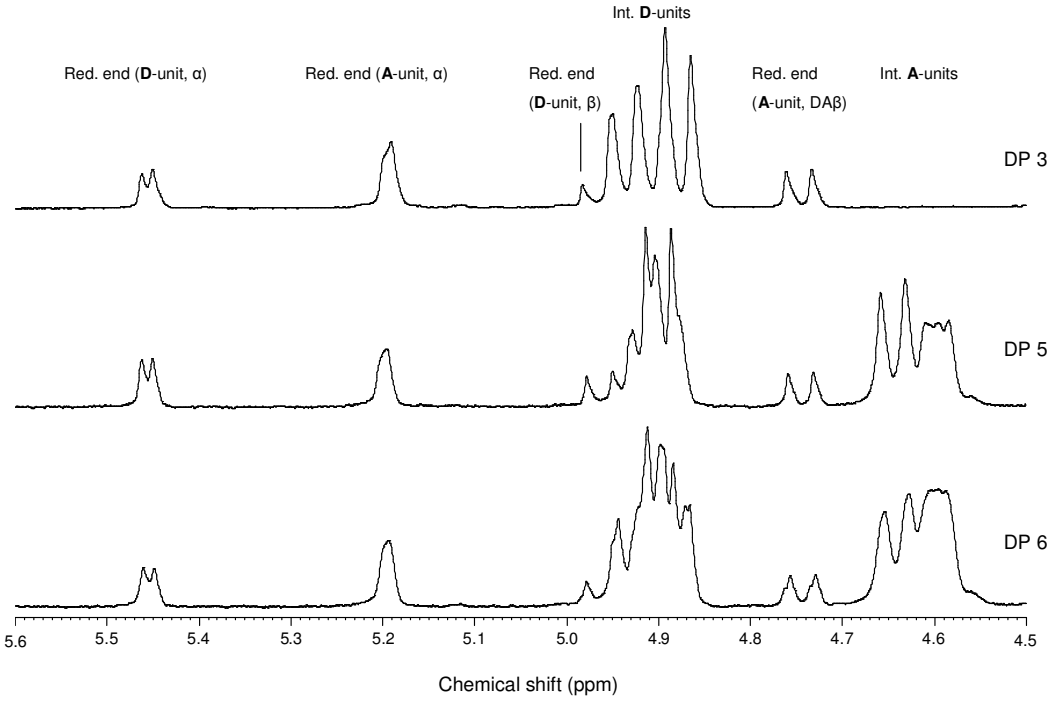
For protein expression, the transformant was grown at 37°C in 2×TY medium containing 100 mg of ampicillin per liter until the OD_{600} reached 0.5, after which gene expression was induced by adding 0.02% (w/v; final concentration) arabinose. After 40 hours incubation at 18°C, cells were harvested by centrifugation and the protein was purified to homogeneity by Ni^{2+} affinity column chromatography using a Ni-NTA Superflow Column (Qiagen, Venlo,

Netherlands), and purity was confirmed by SDS-PAGE. 2 mM $CaCl_2$ and 10% Glycerol were added to all the buffers for protein purification. Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad, USA), with bovine serum albumin as a standard. The protein yield was about 2 mg of pure SaCsn75A per litre of culture of *E. coli*. The protein was stored at -20^{o}C in 20 mM Tris pH 8.0, 20 mM NaCl, 2 mM $CaCl_2$, and 50% Glycerol.

Supporting information

Figure SI 1. ^{1}H-NMR spectra (anomer region) of selected CHOS fractions (DP3, 5 and 6) obtained after extensive degradation (α = 0.21) of a F_A = 0.52 chitosan. Spectra were acquired at 300 MHz, 90 °C and pD 4.2. The reducing end of the α-anomer of an **A**-unit resonates at 5.19 ppm, while the β-anomer of a **A**-unit resonates at 4.742 ppm when the neighboring residue is **D** (present in the spectrum) or at 4.705 ppm if the neighboring residue is an **A** (not present in the spectrum). The α-anomer of a **D**-unit resonates at 5.46 ppm and the β-anomer at 4.97 ppm. The reducing end resonance of **D (**β-anomer) is only partially resolved from the resonance of the internal **D**-units (4.85 - 4.97 ppm) [16, 34]. Internal **A**-units resonate at 4.55 – 4.68 ppm.

Figure SI 1


Int. **D**-units
Red. end (**D**-unit, α)
Red. end (**A**-unit, α)
Red. end (**D**-unit, β)
Red. end (**A**-unit, DAβ)
Int. **A**-units
DP 3
DP 5
DP 6
5.6
5.5
5.4
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6
4.5
Chemical shift (ppm)