ZitR purification

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ABSTRACT

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PROTOCOL STATUS

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MATERIALS TEXT

An untagged, recombinant (S2A, A4R, D8E) form of ZitR protein (145 amino acids, full length) from L. lactis subsp. cremoris strain MG1363 (UniProtKB/Swiss-Prot A2RNS2) was produced and purified as previously described (Llull et al, 2011). Recombinant ZitRMG protein was first over-produced in E. coli strain (BL21(DE3) (pVE8073) at 25 °C. It was then purified in the presence of ZnCl2 in 2-steps by anion exchange chromatography followed by heparin-affinity chromatography. SDS-PAGE analysis revealed the presence of a few high molar mass contaminating E. coli proteins (not shown), which could be eliminated by gel filtration chromatography in 20 mM Tris-HCl (pH 7.0), 200 mM NaCl and 100 µM ZnSO4. At the end of this purification process, we observed a major dimeric form, and a minor tetrameric form (data not shown). The major dimeric form was subsequently studied.

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