

Heterologous expression of fungal lectins in bacterial expression system

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Lectins are proteins which bind reversibly and specifically to carbohydrates. CnSL is a new fungal lectin with an unknown structure and unique characteristics. We assessed heterologous expression of the lectin in a bacterial expression system using a rich growth medium (RGM) and a defined minimal growth medium (MGM) with the goal to prepare a labelled protein for studies of its three-dimensional structure. Two variants of CnSL lectins (CnSLA and CnSLB) were expressed in *Escherichia coli* using the rich autoinduction medium RGM (3,5 h at 37°C, 19 h at 22°C). Alternatively, the CnSLB variant was expressed in the minimal autoinduction medium MGM (2 h at 37°C, 19 h at 22°C). Finally, we analysed the solubility of heterologously expressed CnSL with SDS-PAGE. Successful expression of CnSL in the bacterial expression system was confirmed in all cases. Lectin CnSLB expressed at a higher yield compared to CnSLA. There was no significant difference in protein expression between RGM and MGM, which is encouraging as we are required to use MGM for labelling of the lectin. Both lectin variants were expressed as insoluble inclusion bodies that were solubilized in 8 M urea. In conclusion, no significant difference was observed in heterologous protein expression between RGM and MGM. However, lectins CnSL form inclusion bodies and their purification will require optimization of the refolding process to obtain the proteins with correct tertiary structure. Furthermore, we will explore the possibility of their expression in soluble form in the bacterial periplasm.