Expression and characterization of the GumM , a glycosyltransferase from *Xylella fastidiosa*

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Xylella fastidiosa, causal agent of Citrus variegated chlorosis (CVC), produces an exopolysaccharide called fastidian gum that can be involved in its pathogenicity. Our main aim in this work is the study of the GumM protein, a glycosyltransferase involved in the metabolic path of the fastidian gum. The gumM gene was cloned into the pET28a vector and the recombinant plasmid was transformed into *E. coli* BL21 (DE3) strain, allowing the expression of the GumM in fusion with a histidine-rich peptide. In order to optimize both expression and solubilization of the protein, different concentrations of IPTG and temperature were tested. The best condition for expression of the GumM protein was chosen and the GumM was induced by addition of 1 mM IPTG and incubation was continued overnight at 20^0C. The cells were centrifuged, resuspended in buffer (Tris-HCl 50mM pH 8,0 plus Tween20 0,5%) and disrupted by sonication on ice and subsequently centrifuged at 9000g for 20 min at 4^oC. The supernatant was applied into an Ni-NTA agarose affinity column and the fractions eluted with imidazole were analyzed by SDS-PAGE. On the SDS-PAGE the protein showed one protein band with an apparent molecular weight of the 30 kDa which is similar to the value calculated for the GumM protein. Fractions containing the partially purified GumM His-tag protein were pooled and applied onto a DEAE FF column (GE) equilibrated with 50 mM Tris-HCl buffer pH 8,0 and the protein was eluted as a single peak with a linear NaCl gradient. UDP-glycosyltransferase activity of recombinant GumM is currently being realized.
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