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Title: Exploring methylotrophic yeast Pichia pastoris as a platform for expression of cell surface adhesive proteins

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Abstract: <strong>Introduction</strong>: The C-terminal repeat region of peptidoglycan hydrolase (CPH) of <em>Lactococcus lactis</em> IL1403 produced intracellularly in <em>Escherichia coli</em> was able to attach to the cell surface of lactic acid bacteria (LAB) when added from the outside. Therefore, CPH can be used for binding of a protein of interest to LAB cells when the fusion of CPH to the target protein is incubated with the cells. These cell surface adhesive proteins can confer new functions to LAB without making any genetic modifications in them and are valuable for food and vaccine development. <em>Pichia pastoris</em> has better capability to allow the correct folding of recombinant proteins than prokaryotic hosts e.g. <em>E. coli</em>. However, the glycosylation of the proteins in this yeast may affect their functions. In this study, therefore, we investigated expression of a CPH mutant devoid of potential N-glycosylation sites (CPHM) in <em>P. pastoris</em>. The cell surface binding activity of CPHM was studied and compared with that of CPH produced in <em>E. coli</em>.<br />

**Methods**: <em>cph</em> was cloned into pPICZalphaC (invitrogen) with a hexa histidine tag encoding sequence at its N-terminus. The resultant plasmid was pPIalphaCPH. For construction of CPHM, site directed mutagenesis was performed using a Quickchange kit (Stratagen), three mutagenic primers and pPIalphaCPH as the template. Protein expression in <em>P. pastoris</em> GS115 was induced with methanol at 0.5% v/v. Protein purification was done using nickel chelate affinity chromatography. Protein expression in the yeast and binding of proteins to the cell surface was studied by western blot. <br />

**Results**: CPHM was successfully expressed extracellularly in <em>P. pastoris</em> using alpha-mating factor signal sequence, whereas the native CPH was not produced in this host. Western blot analysis revealed that the apparent molecular size of CPHM was greater than that of CPH produced in <em>E. coli</em>, which is attributed to O-glycosylation. However, CPHM produced in <em>P. pastoris</em> was capable of binding to the cell surface of <em>L. casei</em> NRRL B-441 despite its modification by the yeast, and its dissociation rate constant from the cell surface was 3.5-fold lower than that of CPH produced in <em>E. coli</em>.<br />

**Conclusion**: These results demonstrate the applicability of the constructed domain (CPHM) for the production of cell-surface adhesive proteins in <em>P. pastoris</em>. cell surface adhesion, glycosylation, lactic acid bacteria, peptidoglycan hydrolase, Pichia pastoris

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