

CLONING AND EXPRESSION OF AN ANTI CEA SINGLE CHAIN ANTIBODY FRAGMENT IN THE METHYLOTROPHIC YEAST *Pichia pastoris*

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Introduction

Single chain Fv (scFv) antibody fragments are genetically engineered recombinant fusion proteins in which the variable light (VL) and variable heavy (VH) chain domains are connected by an artificial linker (1). The major reason for which heterologous production of scFv and its derivatives forms is a field in rapid development is the potential application of these small antigen binding proteins if produced at a low cost. Such applications include areas where the specific binding of the antibody fragment is useful, as in tumor therapy and diagnostics, in affinity purification, and as biocatalysts (2, 3).

Here we report the cloning and expression of an active scFv antibody fragment (scFvCEA1904His₆) specific to Carcinoembryonic Antigen (CEA) in the extracellular medium of the methylotrophic yeast *Pichia pastoris*. The exploitation of *P. pastoris* as a tool to produce useful quantities of biologically relevant recombinant proteins has gained special attention due to the existence of well-established fermentation methods and of expression plasmids with very powerful and efficiently methanol-regulated promoters (4).

Experimental procedures

The anti-CEA scFv encoding gene was excised from a bacterial vector in which it was originally cloned (5), and inserted into the *P. pastoris* expression vectors pHIL-S1 (Invitrogen, San Diego, CA) and pPS7 (CIGB, Havana, Cuba), bearing the *Pichia* acid phosphatase 1 (PHO.1) and *Saccharomyces cerevisiae* sucrose invertase 2 (Suc 2) signal sequences, respectively, and the methanol-inducible alcohol oxidase 1 (AOX.1) promoter. These constructs were denominated pPSCEA1904His₆ and pPSHis₆CEA. Competent *P. pastoris* GS115 his4(mut⁺) cells (Invitrogen) were electroporated by application of an exponential decay wave electric pulse of 12 kV/cm, for 4.6 ms, in the presence of BglII-linearized recombinant construct (pPSCEA1904His₆). Several HIS⁺ transformants were analyzed for the presence of the gene product of interest in the extracellular medium and in the cellular pellet. Proteins were detected in SDS-PAGE gels stained with Coomassie brilliant blue, and after electrotransfer to nitrocellulose, using specific rabbit anti scFv polyclonal antibodies, and HRPO conjugated-anti-rabbit

Igs. The biological activity of scFv was monitored with a specific ELISA, where the CEA was coated to the solid phase of 96 microtiter plate wells followed by application of the two-fold diluted samples of culture supernatant before and after induction. Culture supernatant of an induced Human serum albumin (HAS) producer GS115 strain (Invitrogen) as well as from GS115 strain transformed with pHIL-S1 were used as negative controls. Anti scFv-CEA rabbit Igs were used as revealing reagent.

Results and Discussion

The anti CEA scFv fragment had been initially expressed in *E. coli* using several strategies (5, 6) but its yields were limited by a low level of production of the protein to the periplasm or by the inefficiency inherent to refolding of denatured inclusion bodies. To overcome these limitations, the scFv encoding gene was cloned into the *P. pastoris* vectors pHIL-S1 and pPS7 for expression as secreted protein. Two distinct phenotypes among 13 GS115/pPSCEA1904 His⁺ transformants analyzed were obtained: clones exhibiting slow growth in methanol (mut^s), and clones capable of still utilizing methanol (mut⁺). These phenotypic differences were presumed to be due to the interruption of the AOX 1 gene by insertion of the expression plasmid in the case of the mut^s phenotype, and integration of the expression plasmid at an alternative site in the case of the mut⁺ phenotype. Only one clone of each phenotype group secreted proteins of the expected size (27.5 kDa) into the culture medium at high levels (100-200 mg/L). This protein was identified as the scFv by SDS-PAGE and Western-blot. The secreted anti-CEA scFv recombinant protein comprises the vast majority of the total protein in the culture medium, which serves as a first step in purification of the expressed protein (Figure 1). It is a specific advantage of secretion in *P. pastoris*, since the organism secretes only very low levels of native proteins (4). The secreted scFv also binds to CEA in a direct ELISA even when the sample of culture medium is two-fold diluted. These results suggest that secretion of functional scFv fragments by *P. pastoris* can provide a low cost, high yield alternative to current bacterial scFv expression systems.

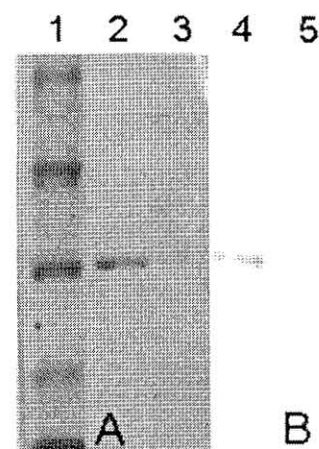


Figure 1. Electrophoretic (A) and Western-blot (B) detection of secreted anti-CEA scFv in the culture medium of a *P. pastoris* mut^sGS115/HIS⁺ transformant. Lane 1: Molecular weight markers (kDa). Lane 2, 4: GS115/pPSCEA1904His₆ (mut⁺) clone. Lane 3, 5: GS115/pHIL-S1 as negative control.

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