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Oral Presentation

Recombinant protein expression system in cold loving microorganisms

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Background

Soluble and functional proteins are of high demand in modern biotechnology. Although many recombinant proteins have been successfully obtained from common prokaryotic and eukaryotic hosts, these systems result to be often unproductive due to the peculiar properties of the protein to be produced. Incorrect folding of the nascent polypeptide chains is one of the main problems occurring during heterologous protein production in bacteria. Since formation of inclusion bodies often impairs the recombinant production of valuable proteins, many experimental approaches have been explored to minimize this undesirable effect [1,2]. Expression of "difficult" proteins has also been carried out by lowering the temperature at the physiological limit allowed for the growth of mesophilic host organisms (between 15 and 18°C for *Escherichia coli*). Lowering the temperature, in fact, has a pleiotropic effect on the folding process, destabilising the hydrophobic interactions needed for intermediates aggregation [3]. On the basis of the above considerations, a rational alternative to mesophilic organisms is the use of naturally cold-adapted bacteria as hosts for protein production at low temperature (even at around 0°C).

Results

The development of a shuttle genetic system for the transformation of the cold adapted Gram-negative bacterium *Pseudoalteromonas haloplanktis* TAC125 (*Ph*TAC125) [4,5] has already been reported. This system has made possible the isolation of constitutive psychrophilic promoters and the construction of cold expression systems for the protein production at low temperatures [6]. The described expression system represented the first example of heterologous protein production based on a true cold-adapted replicon [7]. However, the development of an effective cold expression system with industrial perspectives needs to be finely tuned possibly using *ad hoc* promoters. Physical separation between bacterial growth phase and expression of the desired proteins, in fact, can not only improve the productivity of the entire system but can also play an important role in the production of proteins toxic for the host cells. These goals can only be achieved by using regulated promoters and efficient induction strategies. Recently, using a proteomic approach and exploiting the information deriving from the genome sequence of *Ph*TAC125 [8] we isolated and characterized a functionally active two-component system involved in the transcriptional regulation of the gene coding for an outer membrane porin, that is strongly induced by the presence of L-malate in the medium [9].

In this paper we used the regulative region upstream of the porin gene to construct an inducible expression vector, named pUCRP, that is under the control of L-malate. Performances of the inducible system were tested for both psychrophilic and mesophilic protein production using two "difficult" proteins as model systems. Moreover, an

evaluation of optimal induction conditions for protein production was also carried out.

Conclusion

The inducible expression system was effective in the production of the psychrophilic β-galactosidase from *Ph*TAE79 [10] and the mesophilic α-glucosidase from *Saccharomyces cerevisiae* [11] in a fully soluble and active form.

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