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The LIKE system, a novel protein expression toolbox for Bacillus subtilis based on the liaI promoter

Toymentseva A., Schrecke K., Sharipova M., Mascher T.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

Background: Bacillus subtilis is a very important Gram-positive model organism of high biotechnological relevance, which is widely used as a host for the production of both secreted and cytoplasmic proteins. We developed a novel and efficient expression system, based on the liaI promoter (PliaI) from B. subtilis, which is under control of the LiaRS antibiotic-inducible two-component system. In the absence of a stimulus, this promoter is kept tightly inactive. Upon induction by cell wall antibiotics, it shows an over 100-fold increase in activity within 10 min.Results: Based on these traits of PliaI, we developed a novel LiaRS-controlled gene expression system for B. subtilis (the " LIKE" system). Two expression vectors, the integrative pLIKE-int and the replicative pLIKE-rep, were constructed. To enhance the performance of the PliaI-derived system, site-directed mutagenesis was employed to optimize the ribosome binding site and alter its spacing to the initiation codon used for the translational fusion. The impact of these genetic modifications on protein production yield was measured using GFP as a model protein. Moreover, a number of tailored B. subtilis expression strains containing different markerless chromosomal deletions of the liaIH region were constructed to circumvent undesired protein production, enhance the positive autoregulation of the LiaRS system and thereby increase target gene expression strength from the PliaI promoter.Conclusions: The LIKE protein expression system is a novel protein expression system, which offers a number of advantages over existing systems. Its major advantages are (i) a tightly switched-off promoter during exponential growth in the absence of a stimulus, (ii) a concentration-dependent activation of PliaI in the presence of suitable inducers, (iii) a very fast but transient response with a very high dynamic range of over 100-fold (up to 1,000-fold) induction, (iv) a choice from a range of well-defined, commercially available, and affordable inducers and (v) the convenient conversion of LIKE-derived inducible expression strains into strong constitutive protein production factories. © 2012 Toymentseva et al.; licensee BioMed Central Ltd.

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Keywords

Antibiotic-inducible promoter, Bacillus subtilis, Bacitracin, Cell envelope stress response, LiaIH operon, Protein expression, Two-component system