

High-Level Expression Of Proteins From A Stably Segregating *B. subtilis* Plasmid

Tech ID: 19457 / UC Case 2008-278-0

BACKGROUND

High-level over-expression of commercially important proteins in *B. subtilis* has been difficult to achieve. While there are several different types of *B. subtilis* plasmids that have been used, such as pUB110, pE194, pMTLBS72, or pSM.beta.1, these plasmids are unstable and don't segregate well during cell growth, making them relatively difficult to use for gene expression. During large scale fermentation without antibiotic selection, a significant number of cells (50-99.9 percent) lose their plasmids. Even under antibiotic selection, the bacteria may lose their plasmids unless they have this stable segregation system.

TECHNOLOGY DESCRIPTION

UC San Diego investigators have now discovered a sequence that allows a *Bacillus subtilis* plasmid vector to be stably maintained. It can be used to stably express heterologous proteins in *B. subtilis*, which was not achievable through plasmid expression before. This new vector contains a plasmid stability determinant functional in *B. subtilis* that makes it significantly more stable than any other vector available. The plasmid contains a novel type of DNA segregation system that segregates newly replicated plasmids prior to cell division, even without antibiotic selection. The plasmid vector can replicate in both *E. coli* and *B. subtilis*.

Claim 1: An isolated recombinant plasmid expression vector comprising a polynucleotide encoding a prokaryote-derived actin like protein (ALP) having at least 90% identity to the polypeptide sequence of SEQ ID NO:1, wherein the ALP confers stability on a mobile genetic element when the ALP is expressed in a prokaryotic cell having a mobile genetic element.

APPLICATIONS

The worldwide market for enzymes produced by strains of *Bacillus* is estimated to be greater than \$1 billion, especially for high-level production of cellulases used to produce biofuels or for other industrial processes.

PATENT INFORMATION

Patent 8,636,999

PATENT STATUS

Country	Type	Number	Dated	Case
United States Of America	Issued Patent	8,636,999	01/28/2014	2008-278

RELATED MATERIALS

- Derman AI, Nonejuie P, Michel BC, Truong BD, Fujioka A, Erb ML, Pogliano J. Alp7R regulates expression of the actin-like protein Alp7A in *Bacillus subtilis*. *J Bacteriol.* 2012 May;194(10):2715-24. doi: 10.1128/JB.06550-11. Epub 2012 Mar 16. PMID:22427628
- Derman AI, Becker EC, Truong BD, Fujioka A, Tucey TM, Erb ML, Patterson PC, Pogliano J. Phylogenetic analysis identifies many uncharacterized actin-like proteins (Alps) in bacteria: regulated polymerization, dynamic instability and treadmilling in Alp7A. *Mol Microbiol.* 2009 Aug;73(4):534-52. doi: 10.1111/j.1365-2958.2009.06771.x. Epub 2009 Jul 7. PMID:19602153

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OTHER INFORMATION

CATEGORIZED AS

- **Biotechnology**
 - Genomics
- **Medical**
 - Other
- **Research Tools**
 - Nucleic Acids/DNA/RNA

RELATED CASES

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