



Regulatory Summary

Pfēnex Expression Technology™, a *Pseudomonas*-based system utilizes a natural isolate of *Pseudomonas fluorescens* biovar I, designated MB101, which was obtained from lettuce leaves. It is Gram-negative, aerobic, and nonpathogenic for plants and mammals. This species is ubiquitous in soil, water, and plant environments.

The identity of strain MB101 was independently verified by Dr. I.J. Misaghi, Professor of Plant Pathology, University of Arizona. Phenotypic analyses of strain MB101 involved a variety of biochemical and microbiological characterizations. Genotypic analyses were performed using the 16S rRNA genes of strain MB101 and other selected strains from the genus *Pseudomonas*. Sequence analyses of the three hypervariable regions within the 16S rRNA genes corroborate the findings of the phenotypic analyses that identify strain MB101 as *Pseudomonas fluorescens* biovar I.

Plasmids used for expression of therapeutic proteins in MB101 are self-replicating, non-conjugative, and nonmobilizable derivatives of well-known plasmids RSF1010, pBR322, and pPS10. Antibiotic resistance markers are not present in any of the Dowpharma expression plasmids.

Extensive published studies conducted in mice and rats fed *Pseudomonas fluorescens* biovar I MB101 showed no evidence of pathogenicity or toxicity. A full study report describing the MB101 strain pathogenicity studies was provided to Dr. Stanley Falkow, Professor of Microbiology and Immunology, Stanford University School of Medicine, who is one of the world's leading experts in bacterial pathogenesis. At the completion of his review, Professor Falkow provided a letter, in which he states: "it is my professional judgment the strain of *P. fluorescens* you propose to utilize is nonpathogenic and does not represent an infectious threat of any kind to humans who may come into contact with these bacteria....the microbe you plan to use represents no health risk to your workers or to those who may use it or products derived from it in the future."

Pseudomonas fluorescens biovar I MB101 has been used to produce a food processing enzyme which recently received GRAS notification from the FDA. The safety evaluation of the enzyme protein preparation produced in MB101 is described in a recent publication ("Safety evaluation of an α -amylase enzyme preparation derived from the archaeal order *Thermococcales* as expressed in *Pseudomonas fluorescens* biovar I", by T.D. Landry, et al., *Regulatory Toxicology and Pharmacology* 37 (2003) p. 149-168). This enzyme protein preparation was also tested to investigate its genotoxic potential in a range of *in vitro* and genotoxicity screening assays. These included a mouse lymphoma forward mutation assay, a mouse bone marrow micronucleus test, a chromosomal aberration assay using rat lymphocytes and bacterial mutagenicity assays in *Salmonella* (Ames test). Results of all toxicology studies showed no genotoxicity or mutagenicity.

The American Type Culture Collection (ATCC) classifies strains of *Pseudomonas fluorescens* biovar I as biosafety level (BSL) 1, according to the NIH Guidelines for Research Involving Recombinant DNA Molecules. In addition, *Pseudomonas fluorescens* biovar I MB101 has been safely used to commercially produce a variety of proteins at scales up to thousands of liters, for over 15 years.

A validated immunoenzymetric assay kit for the measurement of *P. fluorescens* host cell proteins (HCPs) is commercially available from Cygnus Technologies, Inc. (Southport, NC). This kit is "generic" in the sense that it is intended to react with essentially all of the HCPs that could contaminate a product manufactured by recombinant expression in *P. fluorescens* independent of the purification process. The assay is very sensitive and precise with an LOD of 40pg/mL, an LOQ of <0.5ng/mL, and an analytical range up to 100 ng/mL in a 2.5 hour simultaneous protocol.

For Further Information

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