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Methanotrophic bacteria: A new platform for recombinant protein production

Microbial surface layer proteins (SLP) have long been thought to represent a source of self-assembling lattices exploitable for applications in nanoelectronics and nano-optics as well as for the development of polymeric surfaces, bio-system fabrications, etc. However, only a few microbial platforms for targeted production of customized SLP-based matrixes have been described. Here we present a new expression platform for protein production which is based on SLP from Methylomicrobium alcaliphilum 20ZR, a methane-utilizing bacterium. The microbe requires a simple mineral media, does not require any growth factors, uses methanol or methane (biogas or natural gas) as its sole source of carbon and energy and can grow in a broad range of pH (6.5-9) and salinity (0.1-9% NaCl). The ability of the strain to produce SLP-matrices comprising conical structures with hexagonal (p6) symmetry was described in early 90s. In spite of the fact that the surface layer comprises up to 10% of total cell protein, the genetics, morphogenesis and function of S-layer proteins have remained elusive. In the current study we explore the mechanisms of S-layer biosynthesis and excretion to develop new strategies for recombinant protein expression. We found that SLPs are exported from the cytosol via a type I secretion system. The system recognizes the C-terminus of the large SLP. Its signal peptide comprises 50 AA and is distantly related to a family of Ca-binding domains. Using this information we tested two approaches for recombinant protein expression: 1) Expression of S-layer fused proteins, which could represent a new strategy for production of proteins immobilized into easy to separate S-layer matrixes; and 2) Expression and secretion of target proteins harboring a short signal peptide. Both approaches were tested using an array of fusions between GFP and lipase (LipL) as test targets. Our results indicate that methanotrophic bacteria represent a promising platform for low-cost production of recombinant proteins, such as industrial enzymes, pharmaceuticals, or optimized protein supplements.

Biography

Marina G Kalyuzhnaya graduated with honors from the Dnepropetrovsk National University, Department of Microbiology, Ukraine in 1994. She earned her PhD in Microbiology in 2000 from the Russian Academy of Sciences, Center for Microbiology and Biotechnology & Institute of Biochemistry and Physiology of Microorganisms. Since 2001 Dr. Kalyuzhnaya has lived and worked in United States, first as a research fellow at the Department of Chemical Engineering, University of Washington, Seattle. In 2006 she became a Research Assistant Professor and then a Research Associate Professor in the Department of Microbiology, University of Washington, where she began to build a research team dedicated to microbial methane conversion. Dr. Kalyuzhnaya is currently an Associate Professor at the Department of Biology and the Viral Information Institute at San Diego State University. Her research interests are focused on improving our knowledge of the methane cycle in nature and developing novel, nature-inspired approaches to improve sustainability of human-made systems and agriculture. Her unique expertise includes microbial genomics and physiology, systems biology and metabolic engineering. She is an author of more than 100 scientific publications, book chapters and numerous patents related to microbial methane conversion.

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