Microbial Genome for Bioengineering

T. Matsunaga
Tokyo University Agriculture and Technology, Tokyo, Japan

Magnetic bacteria synthesize nano-sized bacterial magnetic particles (BMPs) covered with a bilayer membrane of lipid and proteins. BMPs are ultrafine magnetite crystals (50-100 nm diameters) with regular morphology produced by Magnetospirillum magneticum AMB-1. The molecular mechanism of BMP synthesis is a multi-step process which entails vesicle formation, iron transport and magnetite crystallization. To fully understand these complex mechanisms, the whole genome sequence of M. magneticum AMB-1 was analyzed. The genome consists of a single circular chromosome of 4,967,148 base pairs[1]. Most importantly, the iron uptake system, and signal transduction controlling the switching on and off of magnetite biomineralization were analyzed. M. magneticum AMB-1 possesses several iron uptake systems which are common but the encoded gene regulation is unusual. The signal transduction responding to the external environment or to the internal metabolism is comprised of unique and elaborate multi-domain signaling proteins. Proteome analysis of the BMP membrane proteins revealed that the membrane contains a signal response regulator and a number of oxidation-reduction proteins[2]. This comprehensive genome and proteome analysis provides a clearcut resolution of the elaborate regulation of BMP synthesis. Based on theelucidations on the molecular mechanism of BMP formation, methods for construction of functional BMPs were established. Through genetic engineering, functional proteins such as enzyme, antibody, and receptor were assembled onto BMPs. Protein A was successfully assembled on the BMP surface using the iron transporter MagA as an anchor molecule. proteinA-magA gene fusion was transformed into M. magneticum AMB-1 and the extracted recombinant BMPs were used as solid platforms for antibody. We have subsequently reported a fully automated sandwich immunoassay for the determination of human insulin using antibody-proteinA BMP complexes. Seven-transmembrane proteins, G protein-coupled receptors (GPCRs)

were also successfully assembled onto BMPs[3]. GPCRs play central roles in a wide range of biological processes and therefore may provide tremendous pharmaceutical potential. An automated system for single nucleotide polymorphisms (SNPs) detection, immunoassays, and DNA extraction using BMPs has been developed[4]. A fully automated SNP detection system consisting of DNA extraction from whole blood, DNA amplification by PCR, SNP detection by hybridization and fluorescence detection using fluorometer, was constructed. Four DNA fragments in osteoporosis susceptibility gene were then amplified by PCR reaction against same DNA specimen captured on dendrimer-modified BMPs and successfully used for SNP detection.

On the other hand, we have exploited a molecular approach known as the metagenome approachto access genes from the uncultured organisms. It is known that current standard culturing techniques accounts for the culturing of only 1% or less of the bacterial diversity in the environment. A metagenome library was constructed by extracting DNA directly from environmental samples. We focus mainly on the uncultured microorganisms in sponge and coral where a genome library containing DNA fragments from wide diversity of bacteria found within these specimens was constructed. Using the constructed library, they were then subjected to active-based or sequencing-based screening for the isolation of novel genes.Adaptation of the BMP-based automation systems will accelerate the screening process of the target genes from marine environment.

References

1. Y. Okamura, T. Matsunaga et al. (2005) DNA Res. (in press)
2. Y. Okamura, T. Matsunaga et al. (2001) J. Biol. Chem., 276, 48183-48188.
3. T. Yoshino, T. Matsunaga et al. (2004) Appl. Environ. Microbiol., 70, 2880-2885.
4. T. Tanaka and T. Matsunaga (2000) Anal. Chem. 72, 3518.