

Characterization of a Novel Marine Bacterial Flavin-containing Monooxygenase and Its Application to Produce Bio-indigo in Recombinant *Escherichia coli*

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We have cloned a gene from *Methylophaga aminosulfidovorans*, which is a novel marine methylotrophic bacterium. This gene was responsible for generating indigo (the blue pigment). The recombinant plasmid (designated as pBlue 2.2) contained a 2.2 kbp DNA fragment of *M. aminosulfidovorans*. The complete open reading frame (ORF) was 1371 bp long, which encodes a protein of 456 amino acids. The molecular mass of the encoded protein was estimated to be 105 kDa, consisting of homodimer of 54 kDa with an isoelectric point of 5.14. The optimal pH and temperature on its activity were 8.5 and 55°C, respectively. Three characteristic sequence motifs of flavin-containing monooxygenase (FMO) were found: FAD binding domain, FMO-identifying sequence motif, and NADPH binding domain. In addition, its biochemical properties such as substrate specificities and absorption spectra were similar to those of eukaryotic FMO families. Thus we assigned the enzyme to be a bacterial FMO. Bio-indigo was produced in the tryptophan medium (2g tryptophan, 10g NaCl, 5g yeast extract, and 50 µg ampicillin per liter) at 30°C. By batch reaction, the cells produced 920 mg/L of indigo after 20 h cultivation.