CYP102A5, a self-sufficient P450 monooxygenase catalyzing hydroxylation of fatty acids

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The gene encoding CYP102A5, a novel P450 monooxygenase from *Bacillus cereus*, was cloned and expressed in *Escherichia coli* BL21 using pET expression system. It can be easily expressed in Escherichia coli with a high yield. The expressed recombinant enzyme was purified by Ni-NTA affinity chromatography and characterized. This CYP102A5 is a 120 kDa (1065 aa) self-sufficient monooxygenase which belongs to Class III, consisting of an FMN/FAD-containing reductase domain and a heme domain. The deduced amino acid sequence of CYP102A5 exhibits a high level of identity with amino acid sequences of CYP102A1 (60%) from *Bacillus megaterium*, CYP102A2 (75%), CYP102A3 (59%) from *Bacillus subtilis*, CYP102A4 (95%) from *Bacillus anthracis* and CYP102A6 (47%) from *Bradyrhizobium japonicum*. At CO-difference spectra, this enzyme showed a typical Soret band at 448 nm. This enzyme catalyses the oxidation of alkanoic acids (C8 to C17) and unsaturated fatty acids (C18). Substrate binding study showed the tightest binding fatty acid was myristic acid (C14), yielding a Kd value of 7.88 μM. To understand the experimental observation, CYP102A5 homology modeling with CYP102A1 crystal structure was investigated to provide the insight into the catalytic property.