Screening of Novel Cyclosporins hydroxylase Gene Famillies Using microbial Genomics

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Abstract

Cyclosporins are not only known as a hair growth stimulator but also a strong immune suppressor. Several actinomyces such as *Sebekia* and *Pseudonocardia* can hydroxylate the position of N-methyl leucine of cyclosporins. The hydroxycyclosporin has a lower toxicity compared to cyclosporin, although it retains the hair growth stimulating activity. However, the low productivity and low conversion yield are major obstacles that need to be overcome. To commercialize the hydroxycyclosporin, the productivity and the conversion yield need to be greatly improved (5-10 times). In order to achieve this goal, we searched for a novel cyclosporin hydroxylase using genetic methods. *Nocardiopsis dassonvillei* VKM AC-836 strain that show the cyclosporin hydroxylation activity was used to screen for a novel hydroxylase. The genomic library of AC836 was screened by using degenerate PCR product, containing the conserved region of p450 monooxygenase, as a probe. 1.2kbp DNA fragments were isolated and the deduced amino acid sequence of the isolated fragments revealed similarities to known P450 hydroxylase of actinomyces strains. The isolated fragments were cloned in *Streptomyces* expression vector PSE34 and transformed into *S. lividans* for analysis of cyclosporin hydroxylation activity. Detection of cyclosporin hydroxylation activity will allow development of new recombinant strains to improve the conversion yield and the hydroxycyclosporin productivity.