Cloning of styrene monooxygenase gene from a new isolate *Pseudomonas putida* SN-1

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Abstract

Twelve styrene-utilizing bacteria were isolated from a biofilter treating industrial waste gas with styrene as the sole carbon substrate. A gram-negative strain with a high styrene-degrading activity has been identified as *Pseudomonas putida* SN-1 by 16s rRNA analysis. The styrene degradation in SN-1 was catalyzed by a monooxygenase enzyme which converted styrene to epoxystyrene, a potentially important chiral building block in organic synthesis. To construct a recombinant *E.coli,* 4 ORFs encoding styrene monooxygenase (*styAB*), styrene oxide isomerase (*styC*) and phenyl acetaldehyde dehydrogenase (*styD*) were isolated and cloned using the primers that were designed with known consensus sequences. The sequences of the ORFs were determined and compared to the GenBank database by using the BlastP algorithm. Cloning of these genes enabled us to express the single enzymatic activities and biotransformation using the recombinants are under progress.

Table 1. designed primer

Primer	Sequence (5'-3')
styA(forward)	CCCACAAGCTTTAAAAGGAGGAATGAAAAAGCGTATCGGTATTGTTGGTGC
styA(reverse)	AAAAGGTACCACCAGCGGAGCAATAGCGTCAGGCCGCGAT
styB(forward)	AAAAGGTACCAGGAGGAGCGGAGCCCTAACTCCTGGGTGATTCAAA
styB(reverse)	AAAAGAATTCTTCGTTGCGCAATCAATTCAGTGGCAACGC