Molecular Analysis of Phenol-Degrading Microbial Strains

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In an attempt to estimate the occurrence of phenol hydroxylase-related gene sequences we performed a dot blot hybridization assay with DNA from phenol utilizing *Trichosporon cutaneum* R57 strain NBIMCC 2414 and microbial isolates from different wastewaters. The used oligonucletides were homologous to the 5′-end of TORPHD locus (NCBI)-coding phenol hydroxylase in *Trichosporon cutaneum* ATCC 46490 and to the 5′-end of TORCCMLE locus (NCBI)-coding *cis,cis*-muconate-lactonizing enzyme in *Trichosporon cutaneum* ATCC 58094. Two microbial strains, *Escherichia coli* JM 109 and *Lactobacillus acidophilus* ATCC 4356, incapable to degrade phenol were used as negative controls.

We established the presence of hybridization with both used oligonucleotide probes in *T. cutaneum* R57 and *T. cutaneum* ATCC 46490 yeast strains. The experiments implemented with microbial isolates obtained from three industrialized areas in Bulgaria showed that 7 of them may carry sequences hybridizing with a phenol hydroxylase oligonucleotide probe. A subsequent hybridization test for the *cis,cis*-muconate-lactonizing enzyme showed that only 3 of them displayed a positive signal. *Lactobacillus acidophilus* ATCC 4356 and *Escherichia coli* JM 109 strains' DNA used as negative controls in the experiments did not reveal any sequence similarity to the both applied oligonucleotides.

The partial nucleotide sequences of 16S rDNAs of the isolated strains C1 and K1 obtained as PCR products were determined and sequenced. A comparison of these nucleotide sequences with similar sequences in NCBI Data Bank indicated that both C1 and K1 strains are closely related to the genera *Acinetobacter* and *Burkholderia*.

Key words: Trichosporon cutaneum, Dot Hybridization, 16S rDNA