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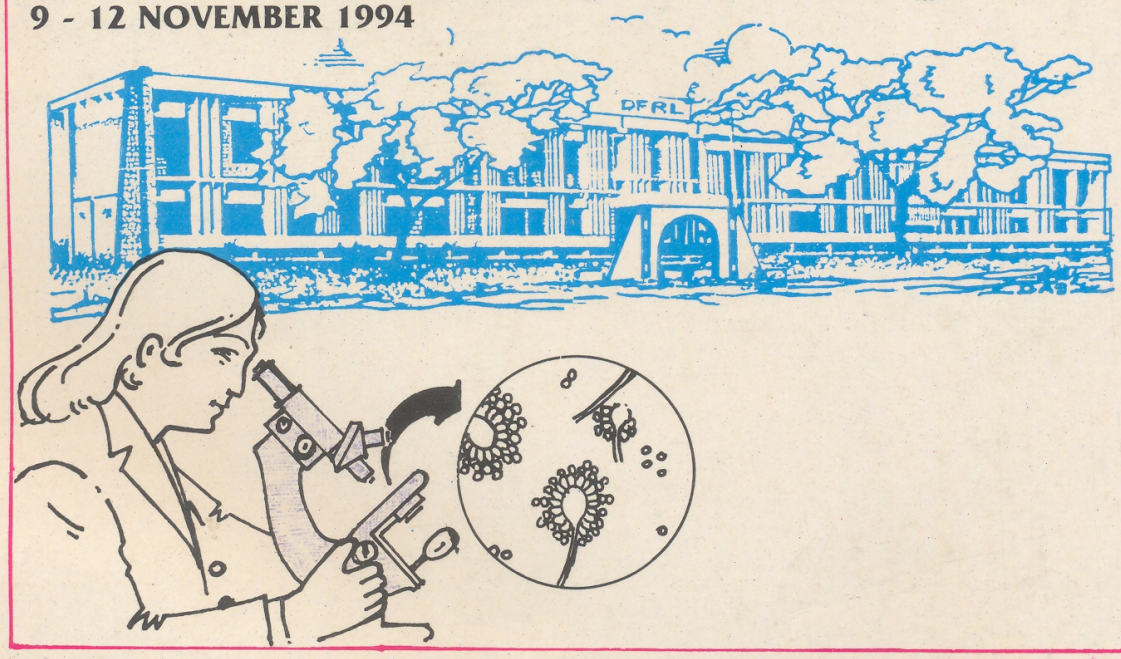

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GE-8

CLONING OF ∝-AMYLASE GENES OF *BACILLUS* SPP. IN *E. COLI* HB101

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∝-Amylase is one of the most important enzymes which finds use in food, pharmaceutical, textile and other industries. Production of this enzyme using native microorganisms is found to be uneconomical. Hence, attempts are made to clone the ∝-amylase genes of two *Bacillus* spp. in *E. coli* with a view to construct recombinant strains by subcloning the genes through a high expression vector in a secretory host later as well as to engineer the protein to improve their catalytic functions. The genomic DNA prepared from *Bacillus laterosporus* (p_3) and a *Bacillus* sp. (M1) was partially digested with Bam R1. The fragments were *in vitro* cloned in the plasmid vector PGEM-4 and transformed into *E. coli* HB101. Several ∝-amylase clones were selected using starch plate containing ampicillin. One of the recombinant strains carrying ∝-amylase genes of strain p_3 designated as B1 21/5 and two strains designated as BM 15/16 and BM 25/20 carrying the gene of strain M1 were selected. All the recombinant plasmids showed an insert carrying the ∝-amylase gene of about 15-16 KB size. All the 3 recombinant strains showed fairly good ∝-amylase activity.

EMB-72

DEGRADATION OF MONOCHLOROBENZOATES BY *PSEUDOMONAS AERUGINOSA* 3mT MOLECULAR GENETIC STUDIES

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Microbial degradation of xenobiotics is gaining importance as an efficient method of treatment of chemical landfills and effluents. Many microbial strains have been isolated and developed recently as chemical scavengers. Many catabolic pathways have been reported to be borne by native plasmids. The performance efficiency of such strains would depend mainly upon their genetic stability and their ability to degrade higher substrate levels. *Pseudomonas aeruginosa* strain 3mT is a laboratory isolate which could degrade high levels of 3-chlorobenzoate (3-CBA 8g/L) and 4-chlorobenzoate (4-CBA, 12g/L) through a chlorocatechol pathway. Strain 3 mT harbours a plasmid of about 25 kb size which was found to be highly stable. Several attempts to cure this strain of the plasmid by continuous growth in rich medium (more than 350 generations) as well as by treatment with acridine orange, ethidium bromide, hexamine ruthenium choloride, mitomycin-C and SDS failed to yield any cured strain. As it was not possible to establish the role of plasmid in the biodegradation of chlorobenzoates by curing, the plasmid was transferred to *E. coli* HB101 by transformation. The transformant acquired resistance to kanamycin and ampicillin as the parent strain 3mT but failed to grow on 3-CBA and 4- CBA. The transformant however, readily degraded benzoate. The results indicate that the plasmid codes for antibiotic resistance and for the benzoate catabolism. It can also be inferred that the enzymes involved in the catabolism of chlorobenzoates are specific and are coded by chromosomal genes.