# GENETIC IMPROVEMENT OF KRAFT LIGNIN DEGRADING BACTERIA *VIA* MUTATION INDUCTION AND PROTOPLAST FUSION

Hassan E. Abd Elsalam[1*], Ashgan Abuo Gabal[2], Samia A. Abdelaziz[3], Abdallah E. Mohamed[1]

[1] Soil and water Technology. Dep., Arid Land cultivation Research institute (ALCDI), City for Scientific Research and Technology Applications (CSAT), New Borg El-Arab, Alex, Egypt, [2]Genetic Dep., Faculty of Agriculture, saba Basha, Alexandria, Egypt, [3]Nucleic Acid Dep., Genetic Engineering Biotechnology Research Institute, CSAT, Alex., Egypt.
*Corresponding author: Fax (+203) 4593423, e-mail zamhelsayed@yahoo.com

**Abstract**

Pulp and paper manufacturing wastes have been recognized as a significant point source of pollution since they have high levels of lignin and their derives, so the main objectives of this study were to overcome the problems, through biodegradation of contaminating water with paper manufacturing wastes (black liquor) via the isolation and identification of various bacterial isolates that appeared high ability to degrade lignin; and studying the effect of physical, chemical mutations and protoplast fusion on the bacterial isolates to increase its biodegradability of lignin. Wild type *Bacillus subtilis subsp. Subtilis, Citrobacter farmeri, Escherichia fergusonii* and *Stenotrophomonas rhizophila* isolated from the pulp and paper manufacturing effluent contaminated soil and water were found to be effective towards lignin degradation. Mutant strains were obtained from the acclimatized species by random mutagenesis using various Ethidium Bromide (EthBr) concentrations (50, 100, 150, 200 µg/ml) and the physical mutagen Ultra Violet (UV) where the bacterial strains cell suspensions were subjected to UV irradiation at 312 nm for different times intervals (3, 5, 10, 15 minutes) and after that mutant strains were tested for lignin degradation. It was found that the maximum degradation rates was achieved by treatment with 100 µg EthBr for strain *Bacillus subtilis subsp. Subtilis* (colour reduction 88.7%, lignin degradation 76.1%) compared to the wild strain (colour reduction 85.4%, lignin degradation 71.4%), and 150 µg EthBr for strain *Citrobacter farmeri* (colour reduction 89.8%, lignin degradation 76.7%) compared to the wild strain (colour reduction 70.1%, lignin degradation 62.2%). Genetic Improvement of lignin degradation by these mutant strains was carried out via Protoplast Fusion aiming to isolate new recombinants (fusants) with high capacity of lignin degradation. Fusant strain had optical density more than parents where it increased from 0.406 and 0.370 to 0.473, colour reduction was enhanced from 88.7, 89.8% for parents to 95.2% for fusant, and lignin degradation was increased from 76.1, 76.6 to 83.2%. Accordingly, the protoplast fusion enhanced growth vigor. In addition, this growth pattern indirectly indicated the enhancement of lignin degradation; this could be directly related to strains improvement.

**Key words**: kraft lignin, *Bacillus subtilis, Citrobacter farmer,* protoplast fusion, black liquor