

## Production of Cellulases by *Trichoderma Spp.* Mutants

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**Background & Objectives:** Cellulose is the most abundant biopolymer in nature. Cellulases, which are mainly three types: endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC3.2.1.91) and beta-glucosidases (EC3.2.1.21) catalyze the hydrolysis of cellulose. Enzymatic hydrolysis of cellulosic biomass are used in different fields, such as production of fuel ethanol from released glucose. *Trichoderma* species are known as the most potent producers of cellulases. Selection of the *Trichoderma* mutant strains showing increased cellulase production.

**Methods:** Seven *Trichoderma* strains were obtained from Persian Type Culture Collection and were cultured on PDA medium. In order to select the best fungi, cellulase activity of native fungi was assayed. Carboxymethyl cellulose sodium (CMC-Na), Avicel and cellobiose were used for endoglucanase, cellobiohydrolase (exoglucanase) and cellobiase (beta-glucosidase) assays, respectively. A sample of 108 conidiospores/ml was treated by 0.2M Sodium Nitrite for 53 minutes at 30°C, while stirred at 150 rev.min<sup>-1</sup>, by which methods, 99.99% mortality was observed. Mutated spores were plated on the Mandel's mineral salts medium and their cellulase activities were judged on the basis of hollow zone diameter surrounding the colonies (H/C). Selected mutants were screened in production medium for their cellulase activity.

**Results:** *Trichoderma parceramosum* (PTCC5140) was selected as the best wild type strain with the highest cellulase activity. After mutagenesis, among 70 obtained mutants, 10 isolates with larger clearing zones were assessed in shake flask cultures. The mutant strain T2 and T5 produced 2.5 and 2.27-fold endoglucanase, respectively. Cellobiohydrolase activity of mutant T10 was 1.3-fold in the comparison with wild type. T9 and T10 produced 3.7 and 4.15 times more beta-glucosidase than the parent strain.

**Conclusion:** Cellulase produced by both parent and mutant strains were evaluated for their efficiency of bioconversion or saccharification. Mutant T10 showed improvement of cellulase production, mainly in cellobiohydrolase and beta-glucosidase activity could be a potential candidate for the bioconversion process.

**Keywords:** Cellulase; Mutant; Nitrous Acid; *Trichoderma parceramosum*