ABSTRACT

The present studies describe the isolation, screening, selection and improvement of a thermophilic fungal strain for cellulase production. A total of eighty-seven fungal strains were isolated from different habitats such as soil, garden compost, decomposing bagasse, textile wastes, herbivore dung, wheat straw and rice straw collected from different localities like Sheikhupura, Khanewal, Sadiqabad and Jacobabad. These isolates were identified as *Aspergillus fumigatus* (18 cultures), *Chaetomium thermophile* var. *dissitum* (02 cultures), *Humicola grisea* var. *thermoidea* (15 cultures), *Humicola insolens* (03 cultures), *Sporotrichum thermophile* (17 cultures), *Talaromyces duponti* (21 cultures) and *Torula thermophila* (11 cultures). These cultures were screened out for the production of cellulase in shake flasks using salt medium (KH_2PO_4, KCl, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, L-asparginine, $CaCl_2$, yeast extract and Sigmacell-101). Of all the isolates, *Humicola insolens* TAS-13 was selected on the basis of better cellulase production as compared to other isolates.

Humicola insolens TAS-13 was improved through UV and chemical mutagenesis like N-methyl-N-nitro-N-nitrosoguanidine (MNNG), nitrous acid (HNO_2), Ethyl methyl sulphonate (EMS) or ethidium bromide (EtBr). Two alternative mutation steps were carried out and mutants were screened for enzyme synthesis. After first step mutation, twenty-six mutants were isolated i.e., seven from UV exposure, eight from MNNG, four from HNO_2, three from EtBr and four from EMS treatment. The five best mutants (TAS_{UV-4}, TAS_{NG-7}, TAS_{HN-4}, TAS_{EB-2} and TAS_{EMS-1}) were further screened on the basis of hyper-cellulolytic ability. These mutants gave 17.36, 41.17, 5.25, 23.07 and 29.07% CMC-ase, 18.87, 30.64, 6.52, 10.41 and 17.30% FP-ase and 19, 61.54, 62.02, 14.28 and 23.07% β-glucosidase, respectively more than their wild strain *Humicola insolens* TAS-13.

When these mutant isolates were further mutated alternatively with the same mutagens under same conditions, a total of thirty-three mutants were picked up as second generation mutants and were tested quantitatively. A mutant strain TAS-$13_{UV-4}{}^{NG-5}$ was selected, which produced 43.19% CMC-ase, 60.15% FP-ase & 59.78% β-glucosidase more as much as first generation mutant TAS-13_{UV-4}. This mutant strain with more efficient cellulolytic system was selected for further optimization of cultural conditions for cellulase production.

The mutant strain TAS-$13_{UV-4}{}^{NG-5}$ and its wild strain were tested in parallel. Seven different media (M-I to M-VII) were evaluated. M-II salt medium reported by Mandel and Reese was proved to be the best for cellulase production by both wild and mutant strain. Addition of different pure cellulosic and lignocellulosic substrates to salt medium was evaluated for the enhanced production of cellulase by both mutant and wild strains. Wild strain produced 1.26 U/ml/min CMC-ase, 0.46 U/ml/min FP-ase and 0.33 U/ml/min β-glucosidase with the addition of wheat straw, while mutant gave 2.81 U/ml/min CMC-ase, 1.58 U/ml/min FP-ase and 1.06 U/ml/min β-glucosidase with cotton seed meal.

The different incubation temperatures (25-60°C) and initial pH (3.5-8.5) of the medium were evaluated for enzyme production by both wild and mutant strains. Both the strains showed best optima of temperature at 45°C and pH at 5.0 to yield high titre of cellulase in the fermented broth. The uses of conidial or vegetative inocula were also optimized. A maximum CMC-ase, FP-ase and β-glucosidase was 1.62, 0.60 and 0.42 U/ml/min by wild and 3.10, 1.34 and 1.97 U/ml/min by mutant strain, respectively when 18 h old vegetative inoculum with 3.5% (w/v) concentration was used.

Addition of seven different nitrogen sources such as ammonium sulphate, urea, peptone, sodium nitrate, yeast extract, meat extract or soybean meal were

used singly as well as in combinations by replacing all nitrogen containing ingredients in M-II salt medium. Wild strain gave 1.53, 0.56 and 0.38 U/ml/min of CMC-ase, FP-ase and β-glucosidase, respectively with the addition of 0.25% meat extract. On the other hand, mutant strain produced 2.86, 1.88 and 1.23 U/ml/min CMC-ase, FP-ase and β-glucosidase, respectively in the presence of 0.1% soybean meal in combination with 0.14% ammonium sulphate.

The scale up studies for cellulase production by wild and mutant strains were also carried out using 7.5 L stirred glass fermentor. Effect of agitation intensity (50-250 rpm) and aeration rate (0.25-1.5 L/L/min), dissolved oxygen (5.0-20%) with agitation cascade (20-250 rpm), controlled and un-controlled pH and different concentrations of vegetative inoculum were studied. Wild strain *Humicola insolens* TAS-13 produced maximum CMC-ase, FP-ase and β-glucosidase (1.62, 0.60 and 0.42 U/ml/min, respectively) at 150 rpm agitation intensity, 0.50 L/L/min aeration rate, 10% dissolved oxygen 56 h after inoculation with 18 h old 3.5% (wet w/v) vegetative inoculum at controlled pH 5.0. Mutant strain *Humicola insolens* TAS-$13_{UV\text{-}4}{}^{NG\text{-}5}$ gave better CMC-ase, FP-ase and β-glucosidase (3.10, 1.34 and 1.97 U/ml/min, respectively) at 0.75 L/L/min aeration rate. All other conditions were same as in wild strain.

Mutant stability with respect to cellulase production was tested for many generations and it was found that developed mutant strain *Humicola insolens* TAS-$13_{UV\text{-}4}{}^{NG\text{-}5}$ was markedly stable for cellulase bio-synthesis under defined conditions.

Crude cellulase enzyme produced by the mutant strain *Humicola insolens* TAS-$13_{UV\text{-}4}{}^{NG\text{-}5}$ was freeze dried in order to obtained concentrated mixture of cellulase enzymes. A total 77.42, 73.88 and 73.60-fold increase in the concentration of CMC-ase, FP-ase and β-glucosidase, respectively was obtained

by freeze drying. Freeze dried cellulase broth was then subjected to determine the pH and temperature requirement for the best catalytic ability. Cellulase produced gave best catalytic activity at neutral pH and 50°C.

The concentrated cellulase was also evaluated for its ability to remove the fuzz fibres/dead cotton, improve the hand finish and effect on pigment and reactive dyeing. Significant removal of fuzz fibres/dead cotton and improvement in hand finish was observed. Changes in fabrics weight was also recoreded after enzyme treatment. There was a 35% increase in depth of pigment dyeing and 5.0% in reactive dyeing along with change in spectral curves, when fabrics were treated with enzyme before dyeing. But, when dyed fabrics were treated with cellulase, then significant removal of dead cotton and biostoning effect on dyed fabrics were achieved.