

## ABSTRACT

The abundance and recalcitrance of chicken feather waste, which is almost 80% composed of keratin, produced as a byproduct of poultry industries can be significantly degraded by ecofriendly and monetary processes involving microbial keratinases. This study focused on the keratinolytic activity of wild and mutant strains of *Mucor mucedo* in vitro as a feather keratin-degrading agent. The production of keratinase in the modified recipe of 1% (w/v) feather substrate fermentation media was optimized using a one-factor-at-a-time (OFAT) technique for purifying and characterizing the stability of keratinase for various pH, temperatures, and incubation periods. The best carbon and nitrogen sources for keratinase production were maltose ( $0.779 \pm 0.007$  U/ml) and yeast extract ( $0.507 \pm 0.007$  U/ml), respectively. The optimized fermentation conditions were 3% substrate, 5g maltose, 4g yeast extract, 1.5g peptone, CaCl<sub>2</sub>, & MnSO<sub>4</sub>, 1g FeSO<sub>4</sub>, 0.75g MgSO<sub>4</sub>, 0.5g KH<sub>2</sub>PO<sub>4</sub>, and 0.25g ZnSO<sub>4</sub>. After optimization of fermentation media components, a 1-liter fermenter inoculated with wild-type *Mucor mucedo* strain was run and maximum yield was obtained ( $1.228 \pm 0.005$  U/ml). Then the wild-type strain was subjected to physical (UV) and chemical (Ethidium bromide) mutagenesis. The maximum survival rate of *Mucor mucedo* colonies for physical mutation was up to 107% and for chemical mutation up to 80-90%. The best keratinase activity was observed by physically mutated fungal keratinase ( $2.987 \pm 0.004$  U/ml). Enzymes extracted from the fermenters inoculated by wild-type, physical, and chemical mutant strains of *Mucor mucedo* were purified to 3.25-fold, 0.57- fold, and 1.07-fold, respectively. The maximum stability of keratinase extracted from the fermenter with wild-type strain was observed at pH 8.0, 50°C for 100 minutes ( $0.814 \pm 0.006$  U/ml). The most stable conditions for the physical mutant were pH 7.0, 30°C for 40 minutes ( $2.427 \pm 0.008$  U/ml), and for the chemical mutant, it was pH 7.0, 70°C for 20 minutes ( $2.348 \pm 0.009$  U/ml). The study showed that *Mucor mucedo* is an efficient producer of keratinase with high keratinolytic activity and it improved its capabilities with physical and chemical mutations. This leads to the possible potential of mutated keratinase for industrial purposes making the environment safe, healthy, and pollution-free.