ABSTRACT

The present study describes the strain improvement through different methods and optimization of cultural conditions for the hyper production of citric acid by *Aspergillus niger* using black strap molasses as a substrate. Fifty different strains of *A. niger* were isolated from various soil samples of district Lahore. Malt extract medium was used for the isolation of *A. niger* and the plates were incubated at 30°C for 3-5 days. In order to maintain the culture of *A. niger*, conidia were shifted to potato dextrose agar medium by providing incubation temperature (30°C) for 3-5 days. Isolates were screened for their potential to synthesize citric acid. The strain that produced maximum amount of citric acid was selected and identified on the basis of cultural characteristics and later by slide culture method. Selected strain was chosen for strain improvement, firstly by UV and then by gamma irradiations. Molasses based medium was used to optimize the fermentation conditions, as an incubation temperature of 30°C, 5.5 pH, sugar consumption 150 g/l and incubation period of 144 h by shake flask fermentation. In order to reduce the heavy metal contents of molasses based medium, EDTA and $K_4Fe(CN)_6$ were used as metal chelators. EDTA and $K_4Fe(CN)_6$ were dispensed in separate medium of various concentrations ranging from 50-300 ppm. An improved concentration of EDTA (150 ppm) and $K_4Fe(CN)_6$ (200 ppm) was further used to optimize the addition time of chelating agent. At optimized concentration and addition time of EDTA (72 h) and $K_4Fe(CN)_6$ (48 h) both of the chelators were added simultaneously into the production medium. However, the results with both of these chelators were not encouraging as they decreased the biosynthesis of citric acid. Citric acid production by EDTA and $K_4Fe(CN)_6$ at 5 % and 4 % inoculum level was 60.5 g/l and 70.5 g/l and yield was 66.92 % and 82.9 %, respectively. A significant increase of about 15.67 fold was exhibited by the mutant strain of *A. niger* (ISO-18-UV-M-10) under optimized conditions.