Purification and characterization of azurin from *Pseudomonas aeruginosa* MTCC 2453 and its effects in breast carcinoma.

ABSTRACT

Azurin reduces the toxic effects of cancer regression. Our investigation used customized methods to focus on synthesizing azurin from different strains of *P. aeruginosa* with apparent homogeneity. We screened the growth of different *P. aeruginosa* MTCC (Microbial Type culture collection) strains (1934, 741, 2453, and 1942) for the synthesis of azurin and for enhanced azurin production. We exposed azurin properties using matrix-assisted laser desorption/ionization (MALDI), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Fourier transform infrared spectroscopy (FTIR), circular dichorism (CD). Our prospective study also revealed which strain of the four had the strongest antiproliferative effect of azurin. *P. aeruginosa* 2453 was the strain that secreted the most azurin and showed remarkable apoptotic effects. Additional studies of possible molecular mechanisms and reactive oxygen species (ROS) generation of *P. aeruginosa* are needed. Our study results showed that MTCC 2453-secreted azurin induces apoptosis in breast carcinoma cells like ZR-75-1 and T47D. This study was also premeditated to evaluate the *in vivo* therapeutic efficacy of azurin from *P. aeruginosa* MTCC 2453 in Dalton's lymphoma (DL) mice model. We found that the DL cells survival rate percentage was 29.69 %, 64.6 % and 88.79 % in 50, 100 and 200 μg/kg body weight of azurin respectively. Investigations of the growth inhibitory mechanism in DL cells were exposed by nuclear fragmentation, and the increased accumulation of DL cells in the sub – G_0/G_1 phases in cell cycle analysis are indications of apoptosis. Further, the cause of apoptosis was revealed by western blot which showed the reduction in the ratio of Bcl-2 and Bax protein expression, the activation of caspases-3 through the release of cytochrome *c* in DL cells. The survival rate of tumor bearing DL mice treated with azurin analyzed by Kaplan Meir method showed an effective anti tumor response as with a dose of 200 μg/kg body weight (an 94.19 % increase in life span (ILS) %).

Key words: *Chromatography, Azurin, Apoptosis, p53, Dalton lymphoma*