Short Communication

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Cytotoxic activity of bioactive metabolites produced by Streptomyces parvulus UFPEDA-3408

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Abstract

The objective of this study was to evaluate the cytotoxic activities of bioactive metabolites from Streptomyces parvulus UFPEDA-3408 against selected human cancer cell lines by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The crude extract and the fractions obtained by partitioning using hexane, methanol and ethyl acetate were tested. The samples were tested for their cytotoxic activity against human laryngeal carcinoma (HEp-2), human lung cancer (NCI-H292) and breast cancer cell line (MCF-7) by using MTT assay. The extract crude and fractions showed prominent cytotoxic activity in vitro against all cancer cell lines. The acetate fraction showed 100% of inhibition at the dose of 50 µg/mL against NCI-H292. The results obtained indicated that products of S. parvulus UFPEDA-3408 have a good cytotoxic activity and could be useful to identify novel molecules with potential anticancer for chemotherapeutic use.

Keywords: Streptomyces; Cancer; Cytotoxicity assay.

Introduction

Cancer is a disease that is characterized by excessive and uncontrolled growth of abnormal cells. This disease is one of the most feared in the world due to lack of effective treatments for most metastatic tumors (Inca, 2015). The search for anti-tumor drugs began in the 40s and since then the natural products research area had significant expansion. With respect to cancer, more than 155 molecules have already been identified and approved for the treatment, 75% are not synthetic molecules and of these 47% are natural products or derivatives of these directly. Microorganisms are also capable of various natural products including antitumor (Newman & Cragg, 2007). Streptomyces, a genus belonging to the actinobacteria phylum, have been studied more each day for being characterized as a major producer of a variety of secondary metabolites, one of the most reported metabolites are antibiotics. However, Streptomyces has been extensively studied for its high production antitumor compounds (Bibb, 2005; Madigan 2010). S. parvulus has been reported as a promising microorganism due its high antimicrobial activity (Silva-Lacerda, et al, 2016), and therefore This work aimed to evaluate the cytotoxic activity of extract and fractions obtained from S. parvulus UFPEDA-3408 against human laryngeal carcinoma (HEp-2), human lung cancer (NCI-H292) and breast cancer cell line (MCF-7).

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Material and Methods

Obtaining crude extract

The strains were cultured in M1 medium (Kawamura et al., 1976) at 37°C for 96 h at 180 rpm. After the fermentation processes, the cell mass was separate to metabolic liquid and extracted with ethanol. The organic solvent extract was then evaporated under reduced pressure to yield an ethanol extract (extract crude). The cell mass was also subjected to a partition process with hexane, ethyl acetate and methanol to separate compounds based on their polarity. The ethanol extract crude and the fractions of partition were used for cytotoxic assay.

Cytotoxic assay

The cytotoxic activity was evaluated using the colorimetric method. These assay is based on the capacity of mitochondrial succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Alley et al., 1988; Mosmann, 1983). Were used the cell lines human laryngeal carcinoma (HEp-2), human lung cancer (NCI-H292) and the cell line human breast adenocarcinoma (MCF-7). The cytotoxic potential of the extract and fractions was tested at a concentration of 50 µg/mL. Doxorubicin (5 µg/mL) was used as positive control. The absorbance was read on an automatic microplate

reader at 560 nm after dissolution of the formazan crystals (Alley et al., 1988). The mean optical density (OD) of the samples was compared with the mean OD of the control. These experiments were performed in triplicate, and the percentage of inhibition was calculated using the program GraphPad Prism 5.0.

Results

Cytotoxic evaluation

The crude extract and ethyl acetate fraction of biomass showed high activity against NCI-H292 strain where the inhibition of cell growth reached to 100%, followed by the methanolic fraction which inhibited 92% of cell growth. The MCF-7 line had their growth inhibited by 86% when in contact with ethyl acetate fraction. The other extracts also showed activity, but with lower inhibition, between 28% to 42% for this tumor. To HEp-2 strain, all the ethyl acetate fraction presented a higher cytotoxic activity with 89% inhibition of the cells. The crude extract and all partitions showed an inhibition of cell growth, with the exception of hexane fraction showed no cytotoxicity for HEp-2 line (figure 1).

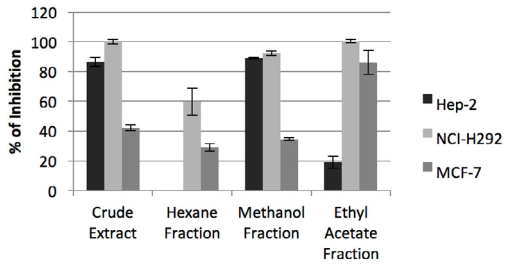

Figure 1 – Cell growth inhibition percentage of extract crude and fractions in three tumor lines tested at a concentration of 50 µg/ml by MTT method after 72 h of incubation.

Discussion and Conclusion

The genus Streptomyces is the best known among the actinobacteria, as a producer of several metabolites with proven bio-efficacy, with wide range of pharmaceutical products such as antimicrobial, anthelmintic, anti-tumor and antiviral agents (Ravikumar et al, 2008). Several studies have reported the activity of Streptomyces metabolites against tumor cells (Sanjivkumar et al, 2016). Ethyl acetate extracts from Streptomyces sp. have been shown to possess cytotoxicity and inhibit cancer cells through a variety of mechanisms including induction of apoptosis, intercalation and binding with cellular DNA, redox-cycling radical formation, and inhibition of topoisomerase (Ignacimuthu et al, 2014).These results obtained in this study suggest that the crude extract and fractions obtained from S. parvulus UFPEDA-3408 have a important place in the process of discovery of new drugs, including chemotherapeutics. For this to be possible, further studies are still being conducted.

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